Universidade de Lisboa

Faculdade de Medicina de Lisboa

CONTRIBUTO DA INFLAMAÇÃO E DE FACTORES DE RISCO CARDIOVASCULAR TRADICIONAIS PARA O DESENVOLVIMENTO DE ATEROSCLEROSE SUBCLÍNICA

ESTUDO COMPARATIVO DE DUAS DOENÇAS REUMÁTICAS INFLAMATÓRIAS SISTÉMICAS

MARIA JOSÉ PARREIRA DOS SANTOS

Doutoramento em Medicina

Especialidade de Reumatologia

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ABREVIATURAS

ACR	Colégio Americano de Reumatologia (*American College of Rheumatology*)
ACPA	Anticorpos anti-proteína citrulinada (A*nti-citrullinated protein antibodies)*
AIM	Marcador informativo de ascendência *(Ancestry informative marker)*
AINE	Anti-inflamatório não esteróide
AIx	Índice de amplificação *(Augmentation index)*
ANA	Anticorpos anti-nucleares *(Anti nuclear antibodies)*
AR	Artrite reumatóide
BANK1	Proteína de sustentação do linfócito B com repetições de anquirina 1 *(B-cell scaffold protein with ankyrin repeats 1)*
BILAG	Índice de actividade do lúpus *(The British Isles Lupus Assessment Group)*
BMI	Índice de massa corporal *(Body mass index)*
CV	Cardiovascular
DAS 28	Score de actividade da artrite reumatóide calculado com base na avaliação de 28 articulações (*Disease activity score 28*)
DNA	Ácido desoxirribonucleico *(Desoxiribonucleic acid)*
EA	Agregação eritrocitária *(Erythrocyte aggregation)*
ED	Deformabilidade eritrocitária *(Erythrocyte deformability)*
EULAR	Liga europeia contra o reumatismo *(European league against rheumatism)*
FR	Factor de risco
FR IgM	Factor reumatóide IgM
GWAS	Estudos de associação genómica de larga escala *(Genome-wide association studies)*
HAQ	Questionário de avaliação de saúde *(Health assessment questionnaire)*
HDL	Lipoproteina de alta densidade *(High density lipoprotein)*

HLA Antigénio leucocitário humano *(Human leucocyte antigen)*

HSP Proteína de choque térmico *(Heat shock protein)*

HTA Hipertensão arterial

ICAM-1 Molécula de adesão intercelular -1 *(Intercellular adhesion molecule -1)*

IL Interleucina

IMT Espessura da íntima-média *(Intima media thickness)*

INE Instituto Nacional de Estatística

IRF5 Factor regulador do interferão 5 *(Interferon regulatory factor 5)*

ITGAM Integrina alfa M *(Intergrin alpha M)*

LDL Lipoproteina de baixa densidade *(Low density lipoprotein)*

LES Lúpus eritematoso sistémico

LTA Linfotoxina alfa

MCP-1 Proteína quimiotática dos monócitos -1 *(Monocyte chemotactic protein -1)*

MHC Complexo *major* de histocompatibilidade *(Major histocompatibility complex)*

MIF Factor de inibição da migração de macrófagos (*Macrophage migration inhibitory factor)*

NO Monóxido de azoto *(Nitric oxide)*

OPG Osteoprotegerina

PAT Tonometria arterial periférica *(Peripheral artery tonometry)*

PV Viscosidade plasmática *(Plasma viscosity)*

RANK Receptor activador do factor nuclear kappa-B *(Receptor activator of nuclear factor kappa-B)*

RANKL Ligando do receptor activador do factor nuclear kappa-B *(Receptor activator of nuclear factor kappa-B ligand)*

SELENA Estudo norte-americano sobre a segurança dos estrogénios no lúpus eritematoso *(Safety of estrogens in lupus erythematosus national assessment)*

SLEDAI Índice de actividade do LES *(Systemic lupus erythematosus disease activity index)*

SLICC-DI Índice de dano do LES *(Systemic lupus international collaborating clinics - damage index)*

SNP Polimorfismo por substituição de um nucleótido *(Single nucleotide polymorphism)*

SRI-50 Índice de resposta SLEDAI 50% *(SLEDAI 2K responder index 50)*

STAT Transdutor de sinal e activador de transcrição *(Signal transducer and activator of transcription)*

TC Tomografia computorizada

TGF Factor transformador de crescimento (*Transforming growth factor)*

TF Factor tecidular *(Tissue factor)*

TM Trombomodulina

TNF Factor de necrose tumoral *(Tumor necrosis factor)*

VCAM-1 Molécula de adesão de células vasculares -1 *(Vascular cell adhesion molecule -1)*

WBV Viscosidade sanguínea *(Whole blood viscosity)*

RESUMO[1]

O objectivo desta dissertação de doutoramento é compreender a relação entre inflamação e aterosclerose usando como modelos o lúpus eritematoso sistémico (LES) e a artrite reumatóide (AR), doenças inflamatórias sistémicas com elevado risco cardiovascular (CV).

O primeiro trabalho visou estabelecer a prevalência de factores de risco CV em mulheres com LES ou com AR sem eventos CV prévios. Constatámos que a hipertensão arterial assim como alterações lipídicas que se traduzem num maior índice aterogénico do plasma são mais frequentes no LES, prevalecendo na AR o excesso de massa gorda, obesidade central e insulino-resistência. Para as alterações da composição corporal a actividade inflamatória *per se* contribui de forma independente.

Nesta mesma amostra estudámos os polimorfismos do promotor do gene do TNF na posição -308 G>A, do promotor do gene da IL-6 na posição -174 G>C e do gene da linfotoxina-α na posição 252 A>G. Os resultados preliminares sugeriram uma associação entre o polimorfismo LTA 252 A>G e a susceptibilidade para AR, que foi confirmada numa amostra maior, constituída por 657 indivíduos caucasianos. O alelo A associou-se a risco aumentado de AR e o genótipo AA a dislipidémia.

Posteriormente avaliámos a relação entre parâmetros hemorreológicos e aterosclerose subclínica detectada por ecodoppler carotídeo. Confirmámos a existência de diversas perturbações hemorreológicas nestes doentes que podem contribuir para a

[1] Resumo até 300 palavras de acordo com o art. 41º do DR nº209, II série de 30 de Outubro de 2006

aterotrombose. A viscosidade sanguínea e a agregação e deformabilidade eritrocitárias correlacionaram-se com a presença de factores de risco CV e com marcadores de inflamação. Demonstrámos que a viscosidade sanguínea e o NO eritrocitário se associam de forma independente à espessura da íntima-média carotídea.

Por último encontrámos perfis distintos de alterações vasculares precoces no LES e na AR. A actividade inflamatória revelou-se determinante para os níveis aumentados de biomarcadores de activação endotelial e para a disfunção endotelial medida pela hiperémia reactiva.

Os resultados obtidos permitem afirmar que um conjunto de parâmetros contribui para a aterosclerose subclínica nas doenças reumáticas sistémicas e revelam a complexa inter-relação entre a inflamação e o risco CV. A identificação de diferenças entre o LES e a AR é crucial para a instituição de medidas conducentes à redução da morbilidade e mortalidade CV.

SUMMARY[2]

The aim of the present dissertation thesis was to understand the relationship between inflammation and atherosclerosis, using as models systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), two systemic inflammatory diseases associated with increased cardiovascular (CV) risk.

In the first part of this thesis we studied the prevalence of CV risk factors in women with SLE and in those with RA free of CV events. We observed that both hypertension and a disturbed lipid profile, translating in a higher atherogenic index of plasma, are more frequent in SLE, while in RA patients excess of fat mass, central obesity and insulin resistance prevail. Inflammatory activity *per se* was an independent contributor to the body composition changes.

Using the same sample, we studied the polymorphisms of the TNF gene promoter at position -308, of the IL-6 gene promoter at position -174 and of the limphotoxin-α gene at position 252. Preliminary results suggested an association between the 252 A>G LTA polymorphism and susceptibility to RA, which was confirmed on a larger sample of 657 Caucasian subjects. The A allele was associated with a higher risk of RA and the AA genotype with dyslipidemia.

Later, we evaluated the relationship between haemorrheological parameters and subclinical atherosclerosis as detected by carotid ecodoppler. We confirmed the presence of several haemorrheological disturbances which could contribute to atherothrombosis. Blood viscosity and erythrocyte aggregation and deformability correlated with the presence of CV risk factors and with inflammation markers.

[2] Up to 300 words in accordance with the 41st article of the DR nº209, II series, 30th October 2006

We showed that blood viscosity and erythrocitary NO are independently associated with carotid intima-media thickness.

Lastly, we found that SLE and RA show distinct patterns of early vascular changes. Inflammatory activity was central to the increased endothelial activation biomarkers and to endothelial dysfunction as measured by reactive hyperemia.

The results we obtained allow us to state that a set of parameters contribute to subclinical atherosclerosis in systemic rheumatic diseases, and they unveil the complex interaction between inflammation and CV risk. The identification of differences between SLE and RA is crucial to the implemenntation of measures leading to a reduction in CV morbidity and mortality.

INTRODUÇÃO

LÚPUS ERITEMATOSO SISTÉMICO

O lúpus eritematoso sistémico (LES) é uma doença inflamatória multiorgânica que envolve a desregulação do sistema imunitário. Caracteriza-se pela presença de anticorpos circulantes específicos para auto-antigénios tais como o DNA, proteínas nucleares e alguns componentes citoplasmáticos, e por uma activação anómala da via do interferão. O LES afecta predominantemente mulheres em idade fértil e tem um pico de incidência que se situa na 3ª década de vida, sendo que 3/4 dos casos são diagnosticados entre os 16 e os 49 anos de idade [1]. A grande diversidade de manifestações fenotípicas e a evolução por surtos, em que períodos de exacerbação intercalam com períodos de remissão, são emblemáticas desta doença.

ETIOPATOGENIA

A causa do LES permanece desconhecida, mas é genericamente aceite que o lúpus resulta de uma interacção entre factores genéticos e ambientais, originando alterações complexas no sistema imunitário inato e adaptativo. Embora a maior parte dos casos de LES ocorra de forma esporádica, a importância da componente genética é patente nos casos de agregação familiar ou na concordância entre gémeos monozigóticos (24-69%), a qual é 10 vezes superior à verificada entre gémeos dizigóticos (2-9%) [2, 3]. Para além da associação clássica com os genes do complexo *major* de histocompatibilidade (MHC), particularmente com o HLA DR2 e o HLA DR3 nos caucasianos [4, 5], e com genes que codificam componentes do complemento [6], é provável que grande parte da susceptibilidade genética esteja

relacionada com genes localizados fora do MHC. Estudos recentes caso-controlo e de associação genómica de larga escala (GWAS) demonstraram de forma inequívoca a associação desta doença com mais de 30 *loci*, o que elucida bem a complexa hereditariedade do lúpus [7-9]. Alguns dos polimorfismos descritos são funcionais e ajudaram a compreender os mecanismos fisiopatológicos do LES. São exemplo disso a associação com variantes dos genes IRF5 ou STAT4, que sustentam a importância central da via do interferão na patogénese da doença, com polimorfismos do ITGAM, que podem ser responsáveis pelas alterações da clearance de complexos imunes, com polimorfismos do BANK1, que podem estar na génese da hiperreactividade das células B, ou com polimorfismos do IRAK1/MECP2, importantes na regulação da transcrição de genes sensíveis à metilação [10]. A identificação de variantes genéticas poderá igualmente num futuro próximo revelar-se essencial para a identificação de novos alvos terapêuticos.

A discordância entre gémeos monozigóticos para o diagnóstico de LES mostra no entanto a importância que elementos que não a genética representam nesta doença. As alterações epigenéticas, que podem resultar de estímulos externos, são fundamentais para a regulação da expressão de genes e estabelecem uma ponte entre factores genéticos e ambientais. A hipometilação do DNA é a alteração epigenética melhor estudada no LES [11] e documentada em vários genes relevantes para esta doença [12]. O estudo da metilação do DNA contribuiu igualmente para uma melhor compreensão do efeito que alguns agentes ambientais, tais como a radiação UV [13] ou certas infecções virais [14] podem ter no desencadear ou na exacerbação do lúpus, e representam ainda o alargar das possibilidades terapêuticas.

A preponderância feminina e a raridade do lúpus antes da puberdade (Figura 1) sugerem a importância do ambiente hormonal, em particular o papel das hormonas sexuais, no despoletar e também na

modulação desta doença. Foi demonstrado de forma clara em modelos animais que a administração de estrogénios exógenos [15] e a hiperprolactinémia [16] aceleram e agravam a doença. Os estrogénios actuam via receptores solúveis intracelulares ER-α e ER-ß e modulam a expressão de genes nas células alvo, mas também podem interagir com receptores da membrana citoplasmática e activar vias de sinalização rápida, não genómicas [17]. A favor da importância dos estrogénios estão igualmente os resultados de estudos caso-controlo que evidenciam o aumento do risco de aparecimento e de exacerbação do lúpus na sequência da toma de anticonceptivos contendo estrogénios [18] e ainda o maior número de exacerbações durante a gravidez [19].

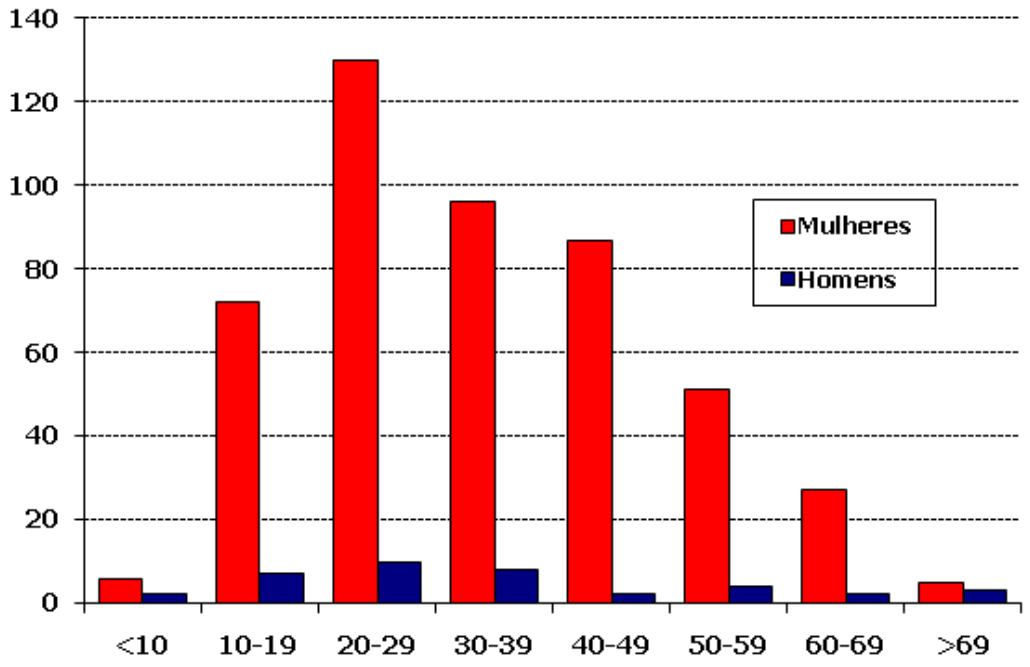

Figura 1- Distribuição da idade de diagnóstico do LES por sexo
Dados referentes a 544 doentes portugueses seguidos regularmente em 5 serviços de reumatologia. Adaptado de Santos MJ *et al* ref [1]

A elevada prevalência de lúpus em indivíduos com deficiência de componentes da via clássica do complemento (C1q, C4, receptor de C2) atesta bem a importância do sistema imunitário inato nesta doença [20]. Duas teorias têm sido apontadas, sendo que uma delas assenta na clearance diminuída de material apoptótico. Em condições normais os corpos apoptóticos, à superfície dos quais são expostos antigénios intracelulares normalmente inacessíveis, são removidos eficazmente pelo sistema imunitário inato, impedindo o contacto dos antigénios com o sistema imunitário adaptativo e a sua consequente activação. Defeitos na remoção destes corpos apoptóticos, como no caso de défice de C1q ou C4, facilitam a activação de linfócitos T e B auto-reactivos e o desenvolvimento de LES. A outra teoria tem por base a hipótese da tolerância: o sistema imunitário inato é importante para a selecção negativa de linfócitos auto-reactivos através da degradação e apresentação de auto-antigénios às células B imaturas, conduzindo à sua anergia. Assim, a existência de deficiências no sistema no sistema imunitário inato facilita a permanência de células auto-reactivas no organismo e consequente desenvolvimento de LES [21].

Entre as numerosas perturbações imunológicas do LES estão anomalias nos linfócitos T e B. A disfuncionalidade dos linfócitos T reguladores (CD4+Foxp3+) contribui provavelmente para a hiperreactividade das células T e B. Esta disfuncionalidade de células CD4+Foxp3+ tem sido extensamente reportada, e com grande probabilidade nem todas as células Foxp3+ dos doentes com lúpus têm uma actividade supressora protectora [22-24].

No LES foram documentados expansão oligoclonal de linfócitos T e defeitos na sinalização e produção alterada de várias citocinas (↓IL-2, ↑INF-α, ↑IL-6, ↑IL-10, ↓TGF-ß), entre outras [25]. Adicionalmente, os linfócitos B hiper-reactivos desempenham um papel patogénico fundamental através da produção de numerosos anticorpos, em

particular contra antigénios nucleares, mas também do citoplasma e da superfície celular. Contudo, o papel das células B no LES vai muito para além da produção de auto-anticorpos, sendo possível induzir lúpus em modelos animais incapazes de produzir imunoglobulinas, desde que estejam presentes células B auto-imunes [26].

ALGUNS ASPECTOS EPIDEMIOLÓGICOS

Existem grandes variações na prevalência e na história natural do LES consoante as áreas geográficas e os grupos étnicos estudados [27-30]. Estas diferenças resultam possivelmente da diversidade genética das populações em causa, mas também de factores ambientais e socioeconómicos. O lúpus é mais frequente nos países do Sul da Europa e no Norte da Austrália do que nos Estados Unidos ou no Japão. Existe alguma evidência a favor de um gradiente crescente Norte-Sul da prevalência do LES na Europa, como testemunham os valores mais baixos encontrados nos países do Norte da Europa (25,4/100 000 e 28/100 000 habitantes na Irlanda do Norte e na Finlândia, respectivamente) quando comparados com os países Mediterrânicos (70/100 000 em Itália, 91/100 000 em Espanha ou 110/100 000 na Grécia) [27, 31]. Não são conhecidos dados exactos de incidência ou prevalência desta doença em Portugal, no entanto existe informação relativa à região Norte do país, com base em dados hospitalares, que permitiu estimar a prevalência do LES naquela zona em 18,8 casos/100 000 habitantes (IC 95% 13,1-27,1) entre o ano de 2000 e 2005 [32]. Este valor é substancialmente mais baixo do que o encontrado noutros países europeus e discordante do gradiente geográfico de prevalência do LES.

A interacção entre factores genéticos e ambientais está patente na prevalência 5-7 vezes mais elevada de lúpus entre africanos e orientais residentes no Reino Unido comparativamente à população inglesa autóctone e também em comparação com a prevalência que se verifica nos países de origem destas populações emigrantes [27].

A origem étnica, o sexo e a idade de início condicionam algumas particularidades clínicas e imunológicas do LES. Os doentes com ancestralidade Sul europeia apresentam mais alterações imunológicas (produção de anticorpos anti-DNA, anti-Sm e anti-fosfolípidos) e úlceras orais mais frequentes. Em contrapartida, os que têm origem na Europa central e do Norte manifestam mais fotossensibilidade e mais lesões cutâneas em geral [33, 34]. Com base no estudo de 6 marcadores identificativos de ascendência (AIM), as úlceras orais associam-se mais especificamente a ancestralidade espanhola e portuguesa [33]. Os doentes do sexo masculino têm maior probabilidade de atingimento de órgãos *major* e de ocorrência de fenómenos trombóticos, enquanto nas mulheres a fotossensibilidade, as úlceras orais e o fenómeno de Raynaud são mais comuns [35-37]. Quando o LES se inicia em idade juvenil o envolvimento renal é mais frequente e a evolução geralmente mais grave do que na doença iniciada após os 50 anos [38, 39] em que a serosite e o atingimento pulmonar são mais comuns [40].

DA INFLAMAÇÃO AGUDA AO DANO IRREVERSÍVEL

Os aspectos patológicos característicos do lúpus incluem a deposição de imunocomplexos, a inflamação e a lesão vascular. Os auto-anticorpos são responsáveis pela formação de complexos anticorpo-

antigénio que mediam o processo inflamatório conducente à disfunção e à lesão dos vários órgãos. A deposição de imunocomplexos nos tecidos leva à activação do complemento e ao aumento da produção de interferão tipo I e de outras citocinas fundamentais à activação dos linfócitos T e B. Nos doentes com LES não só há um aumento da produção de imunocomplexos, como a sua remoção é mais lenta [41].

A gravidade da doença é determinada pela intensidade da resposta inflamatória. A inflamação persistente, não controlada eficazmente, conduz a lesões irreversíveis que se vão acumulando ao longo dos anos de evolução da doença.

A avaliação da actividade inflamatória e do dano irreversível são aspectos que devem fazer parte da monitorização regular destes doentes [42], existindo para o efeito diversos instrumentos disponíveis. Entre os índices válidos, reprodutíveis e sensíveis para medir a actividade lúpica [43], os mais populares são o *systemic lupus erythematosus disease activity index* (SLEDAI), pela facilidade da sua utilização [44], e o *British Isles Lupus Assessment Group* (BILAG), por ser mais informativo [45]. O SLEDAI é um índice global desenvolvido, validado e introduzido na clínica em 1985 e que contabiliza a presença de manifestações clínicas e laboratoriais de actividade lúpica nos 10 dias anteriores [44]. Este índice foi modificado pelos investigadores do estudo "*safety of estrogens in lupus erythematosus national assessment*" (SELENA), mas o SELENA-SLEDAI nunca chegou a ser formalmente validado. Em 2002 foi introduzida a versão revista e validada, o SLEDAI 2K [46]. A pontuação obtida por este instrumento pode variar entre 0–105, e considera-se que a doença está activa se o resultado for ≥4 [47]. Mais recentemente foram desenvolvidos critérios de resposta à terapêutica que conjugam a avaliação pelo SELENA-SLEDAI, pelo BILAG e a avaliação global pelo médico[48], e também critérios de melhoria de 50% no SLEDAI 2K (SRI-50),

destinados principalmente à utilização no âmbito de ensaios clínicos de fármacos [49].

Para avaliação do dano está disponível um único instumento, o SLICC-DI [50]. Este índice quantifica as lesões de carácter permanente e irreversível, acumuladas desde o diagnóstico, independentemente de resultarem da actividade inflamatória, do tratamento, da co-morbilidade ou da combinação destes factores. O SLICC-DI avalia 12 domínios (Figura 2), a pontuação evolui sempre no sentido crescente e pode ascender a 46. Uma pontuação mais elevada reflecte-se numa pior qualidade de vida [51] e num risco de morte mais elevado [52, 53]. Foram identificados diversos preditores de dano entre os quais a idade mais avançada, algumas características clínico-laboratoriais do LES, a corticoterapia crónica, a presença de co-morbilidades e a existência de dano prévio [54-56]. A progressão do dano nem sempre se faz de forma linear e é mais frequente nalguns domínios, como seja o caso do ocular, do musculo-esquelético ou do cardiovascular (Figura 2).

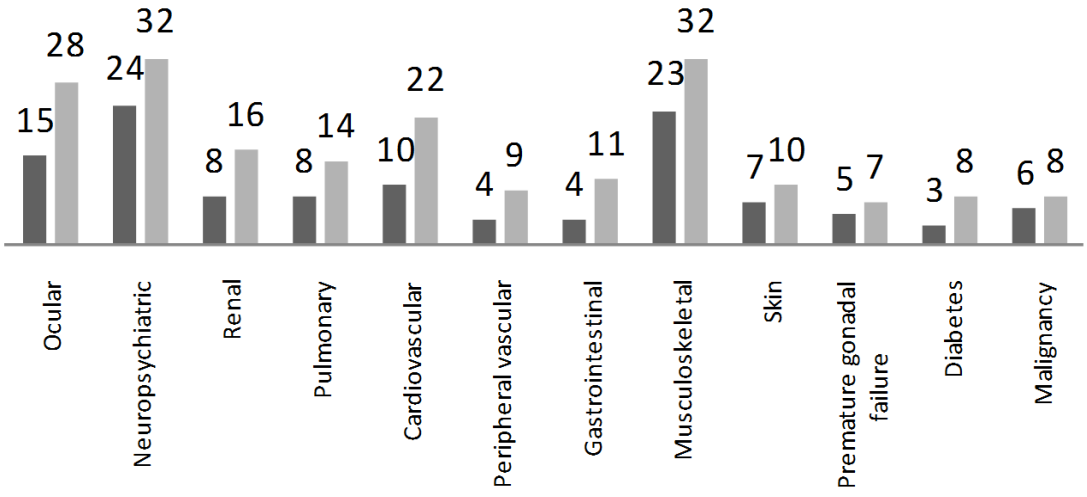

Figura 2 - Número de doentes com dano irreversível medido pelo SLICC na avaliação inicial e após 2 anos

Os domínios que mais contribuíram para a progressão do dano acumulado numa população de 221 doentes com LES foram o ocular, o cardiovascular e o músculo-esquelético. As colunas a preto indicam o número de doentes com dano irreversível presente por domínio do SLICC na avaliação basal e as colunas a cinzento o dano acumulado após 2 anos de seguimento.
Adaptado de Santos MJ *et al* ref [54]

MORTALIDADE E MORBILIDADE CARDIOVASCULAR

Entre as diversas doenças reumáticas que condicionam uma diminuição da esperança de vida, o LES é aquela em que a taxa de mortalidade ajustada é mais elevada, entre 2 a 5 vezes superior ao expectável para o grupo etário e para o sexo [57-59]. É entre as mulheres mais jovens e com doença recente que se situa a taxa de mortalidade mais alta [58]. Este excesso de mortes no período subsequente ao diagnóstico já tinha sido documentado por Merrel *et al* em 1955, num trabalho pioneiro que evidenciou também uma mortalidade de 50% nos 5 anos após o diagnóstico [60]. Desde então a melhoria do prognóstico do lúpus tem sido uma constante [59], em resultado de um diagnóstico mais precoce, de um maior conhecimento e adequada monitorização da doença, mas principalmente por dispormos de terapêuticas capazes de controlar eficazmente a actividade inflamatória (Tabela 1). Actualmente na Europa a sobrevida dos doentes com LES situa-se nos 95% aos 5 anos [61], mas existem grandes disparidades relacionadas com a proveniência étnica, socioeconómica, idade e sexo dos doentes [62]. Apesar desta evolução positiva as mulheres com LES vêem reduzida em cerca de 20 anos a sua esperança de vida estimada à nascença [57].

Data de entrada na coorte	Período de seguimento			
	1970-1978	1979-1987	1988-1996	1997-2005
1970-1978	**13.84**	**4.86**	**3.07**	**3.23**
1979-1987		**6.45**	**3.54**	**3.92**
1988-1996			**4.24**	**3.93**
1997-2005				**3.81**

Tabela 1- Taxa de mortalidade standardizada da coorte de lúpus de Toronto

Análise de 211 mortes ocorridas em 1241 doentes seguidos entre 1970 e 2005, de acordo com a data de entrada e período de seguimento. Adaptado de Urowitz *et al* ref [59].

Com o aumento da sobrevida aumentou também o dano acumulado e alteraram-se as principais causas de morte destes doentes. Enquanto a actividade lúpica e as infecções constituem os principais motivos de óbito nos primeiros anos de doença, mais tardiamente, isto é, 5 ou mais anos após o diagnóstico, a patologia cardiovascular sobressai entre as causas de morte. Este padrão bimodal de mortalidade, descrito por Urowitz há mais de 3 décadas [63], mantém-se válido nos dias de hoje. O contributo da patologia CV para o pico tardio da mortalidade permanece constante mesmo nas observações mais recentes [59, 61, 62]. Apesar do aumento global da esperança de vida dos doentes com LES, a mortalidade CV manteve-se inalterada ao longo dos últimos 25 anos [64]. Na base do excesso de mortes por esta causa estão a aterosclerose acelerada e os fenómenos trombóticos [65]

Os eventos CV não fatais estão também aumentados no LES, variando a sua prevalência entre 1,8% e 27%, consoante a duração do lúpus [66]. O risco de enfarte agudo do miocárdio (EAM) é 2-10 vezes mais elevado comparativamente à população geral. Nas mulheres da coorte de lúpus da Universidade de Pittsburgh com idades compreendidas

entre os 35 e os 44 anos esse risco foi 50 vezes superior ao das mulheres da coorte do estudo *"Framingham offspring"* da mesma faixa etária mas sem LES (RR 52.43; IC95% 21.6-98.5) [67]. Este mesmo estudo mostrou ainda que em 2/3 dos casos o 1º evento CV ocorre em mulheres com menos de 55 anos [67]. Dados de outras populações confirmaram que os eventos CV acontecem em média 10 anos mais cedo nos doentes com LES do que na restante população [68]. Mais recentemente, a avaliação das participantes do *Nurses' Health Study* que à entrada nesta coorte não tinham doença CV nem LES e que durante o seguimento desenvolveram LES, estimou o risco relativo de evento coronário (EAM ou procedimento de revascularização coronária) nas mulheres com lúpus em 2,25 [69]. Este valor, mais baixo que o anteriormente descrito, foi atribuído ao facto da idade das mulheres à data do diagnóstico de LES (52,6±8,6 anos) ser muito mais elevada do que o habitual nesta doença. Também os eventos cerebro-vasculares são mais frequentes no lúpus, ocorrendo cerca de 2 vezes mais nestes doentes do que na população geral [69-71].

Os doentes com LES apresentam não só um risco aumentado de eventos CV fatais e não fatais mas também de aterosclerose subclínica e, ao contrário da que se verifica fora do contexto das doenças inflamatórias, aqui o sexo feminino e a pré-menopausa não conferem protecção contra a doença aterosclerótica. A prevalência de placas ateroscleróticas carotídeas ronda os 17% a 32 % nos doentes sem eventos CV prévios [72, 73]. Estudos caso-controlo, com participantes emparelhados para os factores de risco (FR) CV, mostram que a presença de placas na carótida é 2,4-3,2 vezes superior nos doentes com LES do que nos controlos [74, 75]. Esta diferença é ainda mais notória nos grupos etários mais jovens [74] e nos portadores de anticorpos antifosfolípidos [76]. Os doentes com LES não só têm mais placas ateroscleróticas, como também têm mais placas vulneráveis [77], o que poderá ser relevante para a ocorrência de eventos CV.

Utilizando TC coronária, também em indivíduos sem eventos CV prévios, a presença de calcificações e o score total de cálcio coronário é significativamente mais elevado no LES do que nos controlos [78, 79]. A TC também confirmou que a aterosclerose coronária subclínica surge nestes doentes em idades mais jovens. Os dados relativos ao aumento da espessura da íntima-média são menos consistentes: enquanto alguns autores encontraram uma espessura aumentada nos doentes com lúpus [80, 81], outros não evidenciaram diferenças entre doentes e controlos [74-76].

Numerosos trabalhos comprovaram a importância dos factores de risco CV para o aparecimento da aterosclerose subclínica e para a ocorrência de eventos CV nos doentes com lúpus [65, 72-75, 77-79]. No entanto, a presença de factores de risco CV tradicionais tais como a idade, o sexo masculino, a dislipidémia, a hipertensão arterial, a diabetes ou o tabaco não explica totalmente o excesso de risco CV que estes doentes apresentam [82], da mesma forma que os instrumentos utilizados para a população geral (Framingham, SCORE, Reynolds) não captam o risco acrescido. Factores inerentes à própria doença desempenham um papel importante no risco CV aumentado.

ARTRITE REUMATÓIDE

À semelhança do lúpus, também a Artrite Reumatóide (AR) é uma doença inflamatória crónica sistémica que afecta preferencialmente mulheres. As manifestações clínicas são dominadas pela inflamação crónica de articulações sinoviais, seguindo em regra um padrão articular característico de envolvimento simétrico de articulações periféricas. No entanto, as manifestações sistémicas ou extra-articulares são comuns. Entre estas destacam-se os nódulos reumatóides, o atingimento ocular, pulmonar, cardíaco, vascular e renal.

ETIOPATOGENIA

A AR é uma doença mutifactorial para a qual contribuem factores genéticos e não genéticos. Embora a sua causa não esteja completamente esclarecida, a hereditariedade tem um papel proeminente, quer na susceptibilidade para a doença, quer na modulação da sua gravidade. O estudo de pares de gémeos permitiu identificar concordância para AR entre gémeos monozigóticos em cerca de 15% e nos gémeos dizigóticos em cerca de 4% [83], e possibilitou ainda estimar o contributo da hereditariedade para esta doença em mais de 60% [84]. A associação genética mais forte é com o locus HLA-DRB1 (que contém o epitopo compartilhado) e que explica cerca de 1/3 da variabilidade genética. Recentemente, estudos GWAS confirmaram a importância de mais de 30 *loci* fora do MHC para o risco de AR, embora no seu conjunto expliquem menos de 5% da variabilidade genética desta doença [85].

Entre os factores ambientais, salienta-se o potencial contributo do tabagismo. O tabaco predispõe à transformação da arginina em citrulina, e esta modificação pós-translacional pode tornar diversos péptidos mais imunogénicos e contribuir para a produção de anticorpos dirigidos contra péptidos citrulinados (ACPA) [86]. Os ACPA mostraram uma elevada sensibilidade e especificidade para o diagnóstico de AR [87], são um marcador de prognóstico para doença erosiva e nas artrites iniciais constituem um factor preditivo de evolução para artropatia crónica [88], podendo estes anticorpos estar presentes no soro anos antes da doença. Hipoteticamente será necessário um segundo estímulo para que a doença se manifeste clinicamente. A favor da importância do tabaco na citrulinação de proteínas estão os dados epidemiológicos que mostraram a associação do tabaco apenas com a AR positiva para ACPA. Diversos dados epidemiológicos consubstanciam o sinergismo entre o tabaco e o HLA-DRB1 no risco de AR seropositiva, o qual é 15 vezes superior nos fumadores positivos para o epitopo compartilhado, comparado com os não fumadores negativos para o epitopo compartilhado [89]. Outros estímulos ambientais, como por exemplo a infecção periodontal por *Porphyromonas gingivalis*, podem interagir com genes e despoletar um processo auto-imune [90]. Além dos ACPA e do FR IgM, está documentada a produção de outros auto-anticorpos, sustentando a importância das células B na patogénese da AR.

Na AR estão elevadas diversas citocinas fundamentais na cascata inflamatória, nomeadamente a IL-1, a IL-6 e o TNF. Estas citocinas contribuem para a amplificação e perpetuação do processo inflamatório inicial e a sua produção pelos monócitos e macrófagos a nível da membrana sinovial é determinante para a intensidade da sinovite reumatóide. Estão identificados muitos outros intervenientes neste processo. Recentemente foi evidenciada a participação dos linfócitos Th17 e da IL-17A na fases iniciais da AR [91] e na destruição articular

[92]. Os sinoviocitos, estimulados pela IL-17 e outras citocinas, têm capacidade de proliferar e invadir a cartilagem e o osso subjacentes. A hiperplasia sinovial, a intensidade do infiltrado inflamatório e a proliferação vascular correlacionam-se com a gravidade da AR e com a progressão do dano estrutural [93, 94].

ALGUNS ASPECTOS EPIDEMIOLÓGICOS

A AR encontra-se entre as doenças reumáticas inflamatórias mais frequentes, afectando cerca de 0,5-1% da população mundial. Existem contudo grandes disparidades geográficas, sendo as prevalências mais elevadas observadas entre os índios americanos e do Alasca [95] e as mais baixas em África [96] e na Ásia [97]. Nos países do Sul da Europa a prevalência da AR é intermédia: Grécia 0,68% [98], Espanha 0,5% [99], França 0,31%[100]. Os escassos dados nacionais, baseados num estudo realizado na década de 80 na península de Setúbal, estão em linha com resultados dos outros países da Europa meridional - 0,36% [101]. A doença é mais frequente nas mulheres, que representam cerca de 80% dos indivíduos afectados, e tem um pico de incidência em torno dos 46 anos [102].

LESÃO ESTRUTURAL E CAPACIDADE FUNCIONAL

O diagnóstico de AR significa não somente dor e sofrimento, com a consequente diminuição da qualidade de vida, mas também lesões estruturais irreversíveis resultantes da inflamação articular não controlada. Deixada ao seu curso natural a AR pode ser altamente

incapacitante, com repercussões muito negativas para o indivíduo e para a sociedade. As erosões e a destruição articular surgem precocemente no decurso da doença. A ressonância magnética permite identificar erosões na generalidade dos doentes logo nos primeiros meses [103] e mais de metade apresenta alterações radiográficas após 2 anos da doença [104].

Em resultado da inflamação e do dano estrutural ocorre uma diminuição progressiva da capacidade funcional (Figura 3). Tarefas simples para a generalidade das pessoas, como vestir-se ou comer, podem tornar-se muito difíceis ou mesmo impossíveis para estes doentes. A capacidade funcional medida pelo *Health Assessment Questionnaire Disability Index* (HAQ) é preditiva de absentismo, de perdas económicas, de reforma antecipada e de aumento da mortalidade [105].

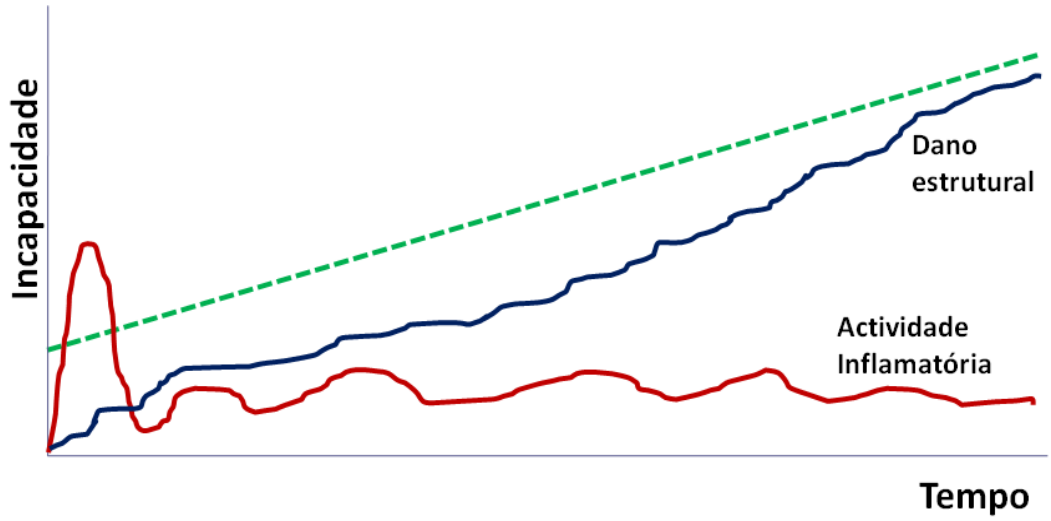

Figura 3 - Progressão da incapacidade no decurso da AR

A AR tem um forte impacto socioeconómico em consequência da impotência funcional e incapacidade laboral que origina, e também do consumo aumentado de recursos de saúde [106]. Para a sociedade implica encargos directos com a doença, mas os maiores custos devem-se à diminuição da produtividade, ao absentismo e às reformas antecipadas. A incapacidade laboral aumenta no decurso da doença, e 10 anos após o diagnóstico cerca de metade dos indivíduos com AR estão incapacitados para o trabalho [107, 108].

MORTALIDADE E MORBILIDADE CARDIOVASCULAR

A esperança de vida está diminuída na artrite reumatóide. A taxa de mortalidade standardizada situa-se em 1,5-1,6 e tem-se mantido estável ao longo dos últimos 60 anos [57, 109]. As causas de morte destes doentes são semelhantes aquelas que se observam na população em geral, mas os óbitos ocorrem 5-15 anos mais cedo do que seria de esperar numa população do mesmo sexo e da mesma idade, mas sem AR. As mulheres com AR vêem reduzida em 7 anos a sua esperança de vida estimada à nascença [57]. As doenças cardiovasculares constituem a causa de morte mais comum, seguindo-se as infecções e a patologia pulmonar, todas elas mais frequentes na AR do que na população em geral. Para além da idade mais avançada, do sexo masculino e da co-morbilidade, uma função física pior [105, 110] e a presença de FR IgM no soro [111] são fortes preditores de mortalidade global mais elevada.

As doenças cardiovasculares são responsáveis por cerca de 40-50% das mortes, um valor que se tem mantido estável ao longo das últimas décadas. Com base em informação proveniente de certidões de óbito, 47,4% das mortes em mulheres com AR no registo de Norfolk

deveram-se a doenças do aparelho circulatório [112]. À semelhança do que sucede no Reino Unido, os dados finlandeses de autópsia apontam as doenças CV como causa imediata ou subjacente a 45% das mortes na AR [113]. O risco standardizado de morte por cardiopatia isquémica é de 1,6 (95%CI 1,46-1,73), e por doença cerebrovascular de 1,52 (95%CI 1,40 - 167) [114, 115]. Da mesma forma que a existência de FR IgM e/ou de ACPA se associam à mortalidade global, são também marcadores de risco de morte CV [111, 116]. Nas mulheres com FR IgM positivos as mortes por doença CV são duas vezes superiores comparativamente às mulheres da mesma faixa etária sem AR [111, 117]. O enfarte agudo do miocárdio ocorre nestes doentes em idades mais jovens e muitas vezes de forma silenciosa [117, 118].

Nas últimas décadas o declínio nas mortes devidas a doenças do aparelho circulatório na população geral tem sido uma realidade em diversos países. Nos EUA verificou-se uma redução superior a 40% em 20 anos (1980-2000), tanto no sexo masculino como feminino, metade da qual se deveu ao controlo eficaz dos FR CV [119]. Entre os homens finlandeses o decréscimo de mortes por doença CV foi ainda maior, em grande parte devido igualmente ao controlo dos factores de risco tradicionais [120]. Em Portugal, de acordo com informação divulgada pelo INE, também se constatou um decréscimo nas mortes por doenças do aparelho circulatório que passaram de 44.5% em 1991 para 31.9% em 2009 (Figura 4).

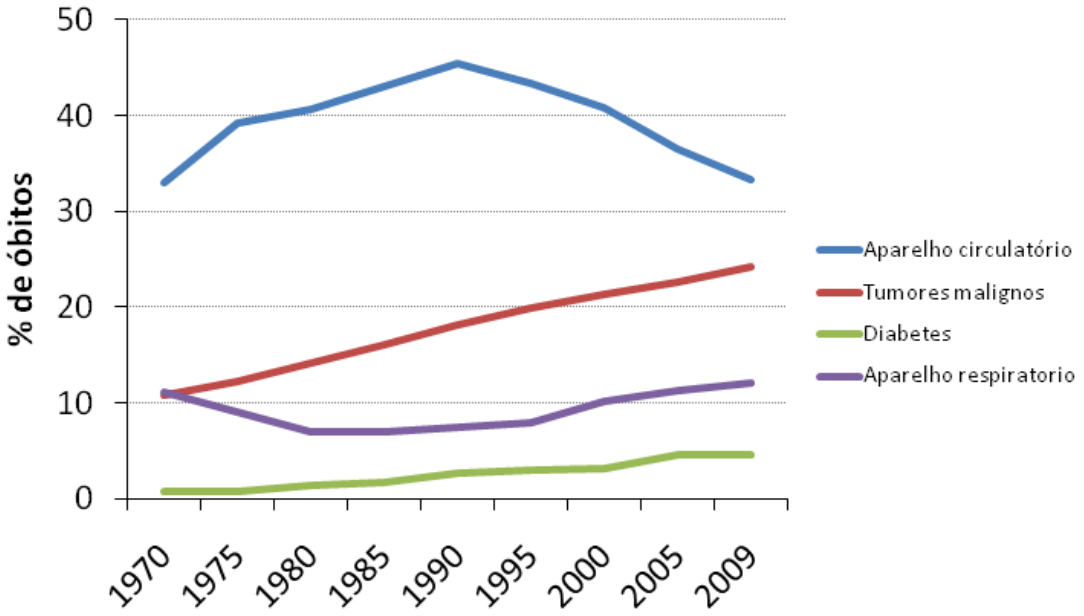

Figura 4 - Evolução das principais causas de morte em Portugal (INE)

Contrariamente ao que se constata na população geral, as mortes por doença CV mantiveram-se inalteradas nos doentes com AR [115], e esta realidade contribui para que o fosso da mortalidade entre esta patologia e a restante população esteja a aumentar (Figura 5) [121].

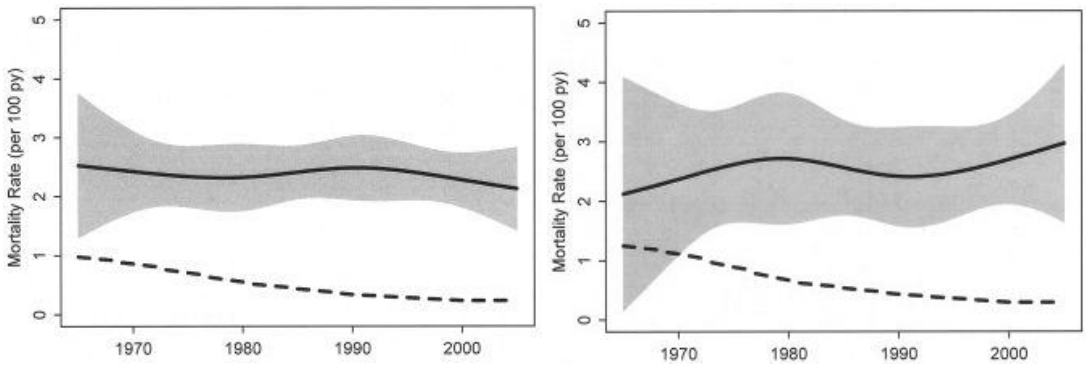

Figura 5 - Evolução da mortalidade nos doentes com AR

Diferença entre a taxa de mortalidade observada (linha contínua) nas mulheres (esquerda) e nos homens (direita) com AR e a esperada (linha tracejada). Adaptado de Gonzalez A *et al* ref [121]

A incidência de eventos CV nestes doentes foi estimada em 3,9 eventos por cada 100 doentes-ano nos Estados Unidos, em oposição aos 0,59 eventos por 100 pessoas-ano calculados para a generalidade da população americana [122]. Está aumentado o risco quer de cardiopatia isquémica, quer de doença cerebrovascular. Em doentes portugueses com AR encontrámos uma incidência de eventos CV mais baixa do que a que se verifica nos Estados Unidos (0,67 por 100 doentes-ano; IC 95% 0,43-1,05) [123]. No entanto, também entre nós os eventos CV são mais comuns na AR do que na população portuguesa em geral, onde a incidência de eventos coronários e de AVC está estimada em 0,14/100 e 0,27-0,3/100 pessoas-ano, respectivamente [124]. Uma meta-análise de estudos de coorte publicados entre 1960 e 2009 calculou a taxa de incidência de EAM na AR em 2,10 (IC 95% 1,52-2,89) e de AVC em 1,91 (IC95% 1,73-2,12) [125]. Para além de mais frequentes, os eventos CV ocorrem cerca de uma década mais cedo na AR [126], sugerindo que esta doença, à semelhança do que sucede na diabetes mellitus, é um factor de risco independente para aterosclerose prematura [118, 127]. Em estudos prospectivos, o risco CV associado à AR e o risco associado à diabetes tipo 2 revelaram-se sobreponíveis [128].

À semelhança do que acontece nos doentes com LES, também na AR está aumentada a aterosclerose subclínica. O risco aumentado de aterosclerose verifica-se mesmo nas fases iniciais da artrite e correlaciona-se com o grau de actividade inflamatória [129]. A prevalência de placas ateroscleróticas é 3 vezes mais elevada nos doentes com AR (44% vs 15%) quando comparada com indivíduos do mesmo sexo, idade, raça e com um perfil de factores de risco CV tradicionais semelhante, mas sem artrite [130, 131]. O aumento da espessura da íntima-média da carótida foi documentado por alguns autores [131, 132], mas não por todos [130, 133].

O excesso da aterosclerose subclínica e de eventos CV fatais e não fatais está bem documentado não só na doença estabelecida, como na

AR precoce [112, 134, 135]. Persiste no entanto alguma controvérsia quanto ao contributo dos FR CV tradicionais. Enquanto alguns estudos não detectaram diferenças na prevalência destes factores entre os doentes com AR que tiveram e os que não tiveram eventos CV [136], outros defendem a existência de mais FR CV entre os doentes com AR, que podem ser responsáveis pela prevalência mais elevada e maior progressão da doença aterosclerótica [137].

Em suma, as causas para o aumento de doença aterosclerótica e de eventos CV fatais e não fatais na AR permanece por esclarecer. Os FR CV tradicionais não explicam totalmente este excesso e a equação de Framingham subestima o risco CV a 10 anos [122, 138]. Parece então haver outros factores relevantes para a aterosclerose precoce nas doenças reumáticas sistémicas, nomeadamente o processo inflamatório subjacente à própria doença.

ATEROSCLEROSE

A aterosclerose e as suas complicações constituem a primeira causa de morte e de perda de vida produtiva no mundo ocidental. Esta doença evolui de forma silenciosa ao longo de décadas, havendo mesmo evidência de alterações vasculares precoces durante o período fetal [139].

Sob o ponto de vista morfológico, a característica central das lesões ateroscleróticas consiste na acumulação anormal de lipoproteinas associada a desorganização, espessamento e alterações fibróticas da parede vascular [140, 141].

Estadios da aterosclerose	**Alterações histológicas**
Espessamento patológico da íntima	A túnica íntima encontra-se espessada, com tecido fibroso laxo, rico em lípidos. Centro lipídico ausente.
Ateroma com cápsula fibrosa	Centro lipídico com cápsula fibrosa espessa. Calcificações raras, dispersas. Infiltrado linfocitário especialmente nas margens do centro lipídico.
Ateroma com cápsula fibrosa fina	Centro lipídico coberto por cápsula fibrosa fina (<80 μm nas coronárias; <200 μm na carótida), por vezes com infiltrado inflamatório.
Ruptura da placa	Ruptura da cápsula fibrosa associada a formação de trombo.
Erosão da placa	Ulceração da íntima com formação de trombo.
Lesões fibrocalcificadas	Lesão com fibrose e extensas áreas calcificadas, resultantes da calcificação distrófica do centro lipídico.
Nódulos calcificados	Lesões intensamente calcificadas, provavelmente um dos estadios finais do processo aterosclerótico.

Tabela 2- Classificação histológica das lesões ateromatosas

Adaptado de Virmani *et al* ref. [141]

Estas modificações estruturais do vaso podem manter-se estáveis e ser assintomáticas durante largos anos (Tabela 2). As placas mais vulneráveis podem complicar-se de fissuras ou ruptura e subsequente trombose, que é responsável pela generalidade dos eventos clínicos [142]. No entanto, algumas fissuras evoluem para cicatrização, em que predomina a proliferação de células musculares lisas, expansão da placa e subsequente agravamento da estenose [143]. A terapêutica antiagregante e hipolipemiante agressiva pode modificar a história natural desta evolução e permitir a cicatrização de fissuras sem que se verifique progressão da estenose [144].

Os factores que contribuem para o aparecimento de alterações precoces e a progressão destas para lesões evoluídas não são totalmente claros, estando presumivelmente envolvidos diversos mecanismos. Deste modo, a aterosclerose é reconhecida presentemente como um processo multifactorial que envolve disfunção endotelial, inflamação, proliferação e migração de células musculares lisas e fibroblastos e alterações da matriz.

De seguida são abordados alguns aspectos etiopatogénicos da aterosclerose, sob a perspectiva da sua possível relação com as doenças reumáticas sistémicas.

ATEROSCLEROSE - UMA DOENÇA INFLAMATÓRIA

Em 1999, Ross define a aterosclerose como sendo uma doença inflamatória [145]. Existe evidência inquestionável a favor do papel que a inflamação desempenha em todas as fases da aterogénese, desde os estadios mais precoces até à ruptura da placa e subsequente trombose.

Na origem das lesões arteriais estão presumivelmente agressões ao endotélio. O endotélio íntegro impede a adesão de células e a formação de trombos. Em caso de lesão (mecânica, química, infecciosa, inflamatória ou outra), as células endoteliais exprimem moléculas de adesão que facilitam a adesão e migração transendotelial de leucócitos. Paralelamente ocorre um aumento da permeabilidade, perda de propriedades anticoagulantes e diminuição da capacidade de produção de NO [146]. Um endotélio mais permeável permite mais facilmente a passagem de lípidos. Os lípidos modificados acumulam-se no espaço extra-celular e favorecem a resposta inflamatória. De facto, o efeito quimiotático que as lipoproteinas oxidadas exercem sobre os monócitos e macrófagos está documentado desde há várias décadas [147].

As lipoproteinas oxidadas, juntamente com outras moléculas quimiotáticas – osteopontina, proteína quimiotática dos monócitos 1 (MCP-1) – medeiam também a migração dos leucócitos para as camadas mais profundas da íntima e contribuem para a proliferação e migração de células musculares lisas [139, 148]. Os monócitos residentes na parede arterial, que são o tipo de leucócitos mais abundantes no vaso, diferenciam-se em macrófagos que fagocitam partículas lipídicas e dão origem às células espumosas. Para além da acumulação de lípidos, os macrófagos desempenham importantes funções pro-inflamatórias através da produção de diversas citocinas como a IL-1ß, o TNF, a LTA, a IL-2, a IL-6, a IL-18 ou o interferão gama [145]. Numa fase incipiente, as alterações histológicas da parede do vaso consistem em agrupamentos de células espumosas que formam as estrias lipídicas [140]. Paralelamente, há recrutamento de linfócitos T e infiltração da íntima por linfócitos T activados (CD4+, HLA-DR+ e IL-2R+) que, embora em menor número que os macrófagos, desempenham presumivelmente funções reguladoras chave na placa aterosclerótica [149]. Os linfócitos B e os mastócitos

são escassos na íntima, mas podem observar-se na adventícia em locais de aterogénese [150].

A inflamação crónica presta um importante contributo para a proliferação das células musculares lisas e para a remodelação da parede vascular. No entanto, os estímulos mecânicos a que o endotélio está directamente exposto, como por exemplo a tensão de cisalhamento ou a pressão arterial, e que comunica à camada média e adventícia de modo a regular a função do vaso, são outro vector da remodelação vascular. Em caso de lesão endotelial, os estímulos mecânicos são percepcionados directamente pelas células musculares lisas, induzindo alterações profundas nas suas propriedades. As células da camada média e também os fibroblastos da adventícia têm capacidade de sofrer transformações fenotípicas rápidas, de proliferar e migrar. Em resposta a agressões, as células musculares lisas produzem matriz extra-celular, incluindo colagénio e elastina, contribuindo assim para a formação da neo-íntima e da placa fibrosa. A progressão das lesões, potenciada por diversos insultos à parede do vaso, é caracterizada por necrose focal e por um processo fibroproliferativo reparador que reduz o lúmen vascular – placa ateromatosa –, altera o fluxo sanguíneo e causa isquémia crónica dos tecidos. Caso ocorra trombose ou embolia da placa há uma interrupção repentina do fluxo sanguíneo que dá origem a um evento isquémico agudo. A trombose é desencadeada pela ruptura da placa e exposição de material trombogénico que conduz à agregação e activação das plaquetas. As placas mais vulneráveis e predispostas a rotura e aterotrombose apresentam também elas um intenso infiltrado inflamatório [151].

No seu conjunto estas observações apontam para o papel relevante que mecanismos inflamatórios e imunológicos representam na aterogénese [139].

DETERMINANTES GENÉTICOS

Factores genéticos podem interagir com o ambiente e modular os processos relacionado com a aterogénese, incluindo a inflamação. Trabalhos recentes utilizando GWAS em grandes grupos populacionais evidenciaram a associação de doença aterosclerótica com diversos polimorfismos de um único nucleótido (SNP), mas o verdadeiro significado deste contributo é ainda incerto [152].

Dada a relevância da inflamação na aterogénese, o contributo de polimorfismos de genes que codificam citocinas pro-inflamatórias tem sido investigado em relação com a predisposição para aterosclerose. Alguns trabalhos documentaram uma associação entre SNP que influenciam os níveis de transcrição de citocinas pró-inflamatória e o risco de eventos CV. Polimorfismos do promotor do gene do TNF na posição -308, particularmente o genótipo AA, associam-se a aterosclerose carotídea e risco aumentado de doença coronária e de EAM [153-157]. Este mesmo polimorfismo associa-se a um risco mais elevado de LES e de AR em algumas populações [158, 159], e em doentes portugueses confere um pior prognóstico [160] e interfere com o padrão de resposta da AR à terapêutica [161].

Polimorfismos do gene da linfotoxina-alfa (LTA) estão associados a níveis séricos de proteína C reactiva aumentados [162] e a risco de aterosclerose, eventos cardiovasculares e acidente vascular cerebral [163, 164]. Em particular, em doentes com AR foi evidenciada a associação entre variantes do gene da LTA e risco de EAM [165].
Existe também alguma evidência a favor da associação de variantes do promotor do gene da IL-6 e um aumento de FR CV e de aterosclerose subclínica na população em geral [166, 167], assim como de eventos CV em doentes com AR[168].

No entanto, os resultados são divergentes consoante as populações estudadas, e persiste a dúvida relativa à real importância destas variações genéticas para a aterogénese [169, 170].

AUTOIMUNIDADE NA ATEROSCLEROSE

A favor da participação da imunidade adaptativa na iniciação e perpetuação da aterosclerose existem dados histopatológicos que revelam a presença de imunoglobulinas e linfócitos T activados na placa ateromatosa [145], mas também várias experiências em modelos animais e observações em humanos. Entre os potenciais antigénios implicados no desencadear da resposta imunológica, encontram-se alguns agentes infecciosos (*Chlamydia pneumoniae, Porphyromonas gingivalis* e alguns vírus) [171], mas também antigénios endógenos, como a proteína de choque térmico (HSP), a ß2-Glicoproteina I ou a LDL oxidada.

Nos modelos animais salientam-se as observações em ratinhos com imunodeficiência severa combinada que apresentam reduzida susceptibilidade para aterosclerose e em que essa susceptibilidade pode ser revertida com a transferência de linfócitos T CD4+ [172]. Também a imunização com determinados autoantigénios é capaz de desencadear um processo imunológico e modular a progressão das lesões ateroscleróticas [173, 174]. Analogamente, a depleção de linfócitos B revelou-se protectora, enquanto a transfecção de linfócitos B agravou significativamente a aterosclerose em modelos murinos [175, 176].

Muitos dos linfócitos T presentes na placa aterosclerótica são reactivos contra a LDL oxidada. Nos doentes com LES, os níveis de anticorpos

anti-LDL oxidada e anti-lipase de lipoproteinas correlacionam-se com a actividade da doença e com a espessura da íntima-média carotídea, o que sugere igualmente o envolvimento de linfócitos B neste processo [177]. No entanto, a relevância destes anticorpos para a aterosclerose na população geral permanece controversa [178, 179].

HEMORREOLOGIA E ATEROTROMBOSE

A doença aterosclerótica manifesta-se frequentemente por eventos trombóticos coronários, cerebro-vasculares ou periféricos agudos. Os factores hemostáticos e hemorreológicos estão manifestamente associados a eventos vasculares isquémicos na população geral e alguns trabalhos mostraram inclusivamente que o contributo das características reológicas do sangue é idêntico ou superior ao dos factores de risco CV tradicionais [180]. Ao favorecerem a trombose e promoverem a lesão endotelial e o espessamento difuso da íntima, as características reológicas do sangue surgem assim como um elemento de grande importância para a aterotrombose.

Desde os anos 70 que diversos estudos identificaram os níveis elevados de fibrinogénio como um factor de risco independente não apenas para doença coronária e cerebro-vascular, mas também como um factor associado a mortalidade mais elevada [181]. Os níveis de fibrinogénio aumentam em resposta à inflamação, como é o caso do LES e da AR, afectando de forma significativa as propriedades reológicas do sangue, a coagulação e a agregação plaquetária, o que sugere que seja esta uma das vias pela qual o risco CV está aumentado na inflamação.

A viscosidade sanguínea é um parâmetro reológico global que depende largamente do hematócrito, da deformabilidade eritrocitária e do fibrinogénio plasmático [182], e que pode contribuir para o desenvolvimento de aterosclerose através do efeito mecânico na parede arterial e da lesão endotelial [183]. Com efeito, na população geral a viscosidade sanguínea está associada a diversos factores de risco CV e ao aumento da espessura da íntima-média [180].
A nível microcirculatório, o aumento da agregação e a diminuição da deformabilidade dos eritrócitos contribuem para disfunção microvascular [184-186].

Embora o contributo das perturbações hemoreológicas para a aterotrombose esteja bem documentado e a influência da inflamação para a alteração destes parâmetros plausível, a informação relativa ao que sucede nas doenças reumáticas é muito escassa.

ADIPOCINAS E ATEROGÉNESE

A inflamação crónica pode favorecer alterações da composição corporal que incluem o aumento da gordura [187]. O excesso de tecido adiposo, em particular da gordura visceral, contribui para o risco cardiovascular acrescido [188].

O tecido adiposo é visto actualmente como um órgão activo que produz e segrega numerosos mediadores, genericamente designados por adipocinas ou adipocitocinas. Estas moléculas bioactivas regulam o metabolismo dos glícidos e dos lípidos, actuam na resposta inflamatória, na regulação imunológica e também na angiogénese. As adipocinas surgem assim como um potencial elo de ligação entre o tecido adiposo, a inflamação e a aterogénese (Figura 6) [189], e são

apontadas por alguns como possíveis marcadores de risco cardio-metabólico.

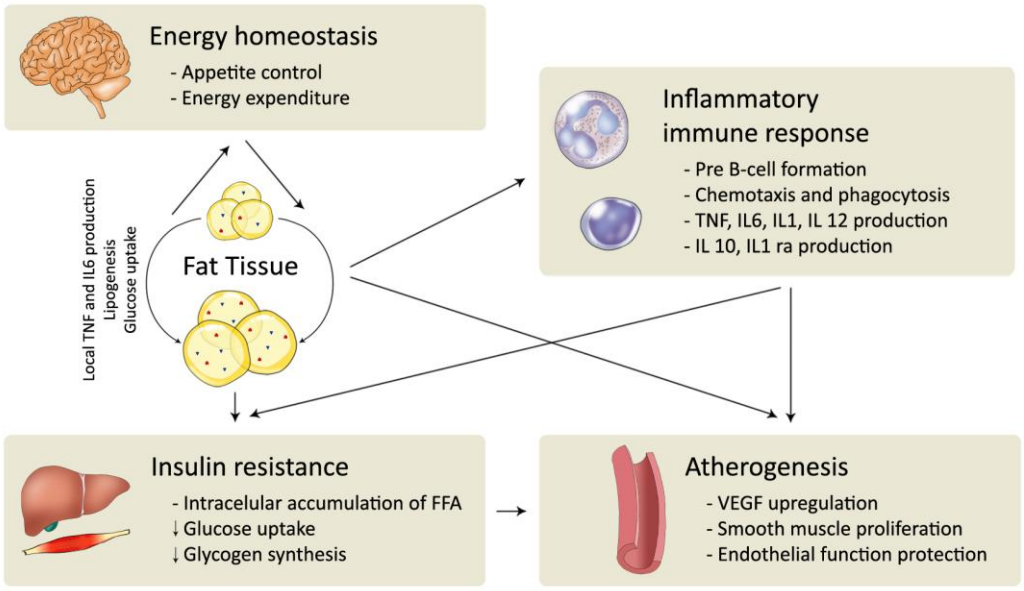

Figura 6 - Relação entre tecido adiposo e aterosclerose

Através da produção de adipocinas, o tecido adiposo modula activamente diversas vias envolvidas no balanço energético, na inflamação e resposta imunitária, na insulino-resistência e na aterosclerose. As adipocinas também actuam como um factor autócrino/paracrino com efeitos na adipogénese, lipogénese e *uptake* de glucose. Os adipócitos produzem proteína quimiotática dos monócitos (MCP-1) que promove a infiltração do tecido adiposo por células mononucleadas, responsáveis pela maior parte da produção local de TNF e IL-6. Adaptado de Santos MJ *et al* ref [189]

Estão identificadas mais de 50 adipocinas, onde se incluem a leptina, a adiponectina, a resistina, a vasfina, a grelina ou a visfatina. Os principais efeitos conhecidos de algumas delas estão resumidos na Tabela 3.

A leptina e a adiponectina são as adipocinas melhor estudadas na sua relação com a aterosclerose. Embora existam alguns dados

divergentes [190], a maior parte dos estudos mostra um efeito pró-aterogénico da leptina, enquanto a adiponectina tem sido apontada como tendo um efeito atero-protector [191, 192]. Esse possível efeito anti-aterogénico foi recentemente corroborado pela associação negativa entre os níveis séricos de adiponectina e a espessura da íntima-média num estudo de grandes dimensões [193].

Curiosamente, não foi encontrada relação entre os níveis séricos de leptina e de adiponectina com a aterosclerose subclínica medida pela deposição de cálcio nas artérias coronárias em doentes com LES ou com AR [194, 195]. Contudo, os níveis de leptina são mais elevados nas mulheres com lúpus que têm placas ateroscleróticas carotídeas [196]. A relação de outras adipocinas com aterosclerose subclínica ou eventos cardiovasculares é nesta altura ainda incerta.

	Leptina	**Adiponectina**	**Resistina**	**Visfatina**
Insulino-resistência	↓	↓	↑	↓
Metabolismo	↑	↑		
Apetite	↓	=;↑(?)		
Adipogénese	↓	↓	↓ (?)	↑
Inflamação	↑	↓	↑	↑ (?)
Aterogénese	↑	↓	↑ (?)	↑ (?)

Tabela 3 - Principais efeitos de algumas adipocinas

↑ aumenta; ↓ diminui ;(?) efeito possível, mas incerto; = sem efeito. Adaptado de Santos MJ *et al* ref [189]

METABOLISMO ÓSSEO E CALCIFICAÇÃO VASCULAR

As calcificações vasculares são um achado frequente na aterosclerose evoluída, assim como em diversas doenças metabólicas. As calcificações da íntima associam-se caracteristicamente à placa de ateroma, enquanto as calcificações da média (doença de Mönckeberg) encontram-se mais frequentemente nos diabéticos, na insuficiência renal crónica e nos indivíduos idosos. A calcificação não resulta apenas da deposição passiva de cálcio e fósforo na parede vascular. As células vasculares, em condições normais, expressam inibidores da calcificação e também outras moléculas reguladoras da mineralização. Caso ocorra lesão da parede, a capacidade de inibição natural da calcificação diminui e pode mesmo perder-se. Entre os mediadores de calcificação identificados na parede dos vasos estão a proteína Gla da matriz, a osteopontina, as proteínas morfogénicas do osso, o RANKL e a osteoprotegerina (OPG). É também possível que mediadores inflamatórios presentes no soro promovam a expressão de moléculas fundamentais no processo de calcificação da parede vascular.

Recentemente foi identificada uma conexão entre a sinalização RANK/RANKL/OPG, essencial no equilíbrio ósseo, e a calcificação vascular. Sob o ponto de vista epidemiológico não é surpreendente esta relação entre as citocinas de remodelação do osso e calcificação do vaso, pois as calcificações vasculares e a perda de massa óssea ocorrem frequentemente em paralelo e partilham diversos factores de risco (idade, inflamação, corticoterapia, insuficiência renal crónica ou carência de estrogénios) [197]. Contudo, o papel exacto destas citocinas na doença vascular permanece controverso. Enquanto estudos *in vitro* e em modelos animais sugerem que a OPG inibe a calcificação vascular [198], os dados clínicos paradoxalmente mostram níveis aumentados de OPG em associação com disfunção endotelial, calcificação vascular, eventos CV e aumento da mortalidade [199]. A

expressão de RANK e RANKL pelos leucócitos do sangue periférico está aumentada em doentes com angina instável, assim como nos monócitos/macrófagos de trombos removidos de doentes com EAM e ainda na placa ateromatosa em modelos animais, apontando para um provável papel do RANK/RANKL na instabilidade da placa [200]. É importante relembrar que a AR e o LES interferem com o metabolismo ósseo e influenciam o sistema RANK/RANKL/OPG [94, 201], e que em doentes com AR os níveis de OPG se correlacionam com o score de cálcio nas coronárias [202].

A vitamina D poderá representar um outro elo entre o metabolismo ósseo e a doença CV. Para além da sua acção no metabolismo fosfo-cálcico, a vitamina D interage com o sistema imunitário inibindo a proliferação de linfócitos T, particularmente dos Th1, a produção de IL-2 e interferão gama e a produção de anticorpos pelos linfócitos B. Estas características conferem-lhe propriedades anti-inflamatórias e anti-proliferativas que podem ser também cardio-protectoras. Os níveis séricos reduzidos desta vitamina associam-se à presença de factores de risco CV, de aterosclerose subclínica e de eventos CV [203]. Nos doentes com LES e nos doentes com AR é comum a carência de vitamina D [204], em parte devido ao tratamento com corticosteróides. Há a acrescentar que os doentes com lúpus evitam a exposição solar e consequentemente a síntese de colecalciferol ao nível da pele está reduzida [205].

FÁRMACOS ANTI-REUMÁTICOS E RISCO CARDIOVASCULAR

Ao interferir com o processo inflamatório, com os factores de risco ou ambos, alguns dos medicamentos usados no tratamento do LES ou da AR podem ter repercussões no risco cardiovascular.

Os anti-inflamatórios não esteróides, frequentemente utilizados por estes doentes durante longos períodos, podem aumentar os valores tensionais e a incidência de eventos CV [206]. O efeito deletério dos corticóides ao promoverem alterações nos padrões lipídicos, aumentarem a tensão arterial e a insulino-resistência é contrabalançado pela sua acção anti-inflamatória potente, e o resultado líquido deste balanço na aterogénese não é necessariamente negativo quando utilizados em doses baixas e por curtos períodos de tempo [207]. Da mesma forma, o metotrexato, causando hiperhomocisteinémia, pode ser lesivo para o endotélio e teoricamente contribuir para o aumento da aterogénese. Contudo, este e outros fármacos modificadores das doenças reumáticas, como por exemplo os antipalúdicos, associam-se de forma consistente a uma redução da disfunção endotelial, da aterosclerose subclínica, de eventos CV e da mortalidade em geral [208-212]. Mais recentemente também foi constatado o benefício dos fármacos bloqueadores do TNF na redução de eventos CV em doentes com AR [213, 214].

DETECÇÃO DAS ALTERAÇÕES VASCULARES SUBCLÍNICAS

Diversos métodos permitem avaliar alterações vasculares funcionais ou alterações estruturais em indivíduos sem doença aterosclerótica clinicamente evidente.

O endotélio é o principal regulador da parede vascular, fundamental para a manutenção do equilíbrio entre vasoconstrição e vasodilatação, entre estimulação e inibição da proliferação e migração das células musculares lisas e entre trombose e fibrinólise. A função endotelial encontra-se comprometida nos indivíduos com doença aterosclerótica estabelecida [215], mas as alterações da função endotelial são também um marcador precoce de doença vascular, podendo preceder as alterações estruturais da parede arterial [216]. A presença de factores de risco CV é um forte preditor de disfunção endotelial [217], e a inflamação subclínica correlaciona-se com alterações do endotélio [218]. Estas alterações são potencialmente reversíveis com medidas farmacológicas (estatinas, colestiramina, inibidores do enzima de conversão da angiotensina ou agentes hipoglicemiantes) e não farmacológicas (dieta ou exercício físico) [219, 220].

Os métodos para determinação da função endotelial baseiam-se na medição da resposta vascular a estímulos que aumentam a produção de NO. A avaliação pode ser feita em diversos leitos vasculares, sendo que a resposta da circulação coronária à acetilcolina medida por angiografia é considerada por muitos o *gold-standard* [215]. A função endotelial é passível de avaliação não invasiva através do estudo da vasodilatação mediada pelo endotélio. Dada a natureza sistémica do processo aterosclerótico existe uma boa correlação entre a disfunção endotelial medida no antebraço e a disfunção a nível coronário [221]. A vasodilatação reactiva pode ser avaliada por tonometria arterial periférica (PAT), neste caso a nível dos dedos, com a vantagem teórica desta avaliação ser independente do observador. Também a

vasodilatação medida por PAT mostrou boa correlação com a presença de factores de risco CV [220], de doença coronária [222] e com a capacidade para predizer eventos CV futuros [223]. A medição é feita de forma contínua durante um período basal (10 minutos), durante isquémia induzida por uma braçadeira insuflada para valores supra-sistólicos (5 minutos) e durante 10 minutos adicionais após desinsuflar a braçadeira. O índice de hiperémia reactiva (RHI) é calculado dividindo a amplitude do sinal após desinsuflação pela amplitude basal do sinal (Figura 7).

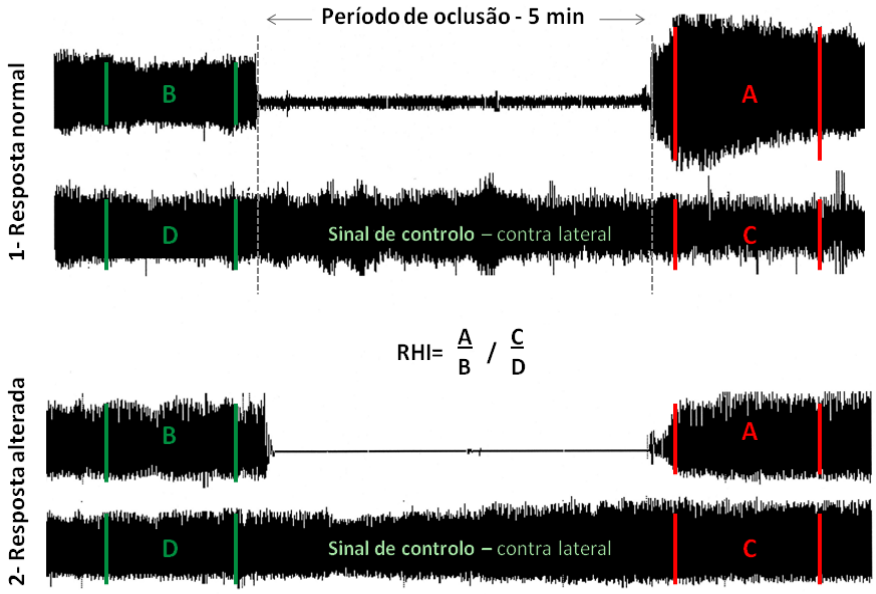

Figura 7 - RHI obtido por PAT

O endotélio intacto (1) responde à isquémia com hiperémia reactiva, mas em caso de lesão essa vasodilatação não se verifica (2). O índice de hiperémia reactiva (RHI) é a razão entre a amplitude do sinal após isquémia (A) e a amplitude basal (B) ajustado para as variações do sinal de controlo medido no membro contralateral (C/D).

Com esta técnica é ainda possível obter o índice de amplificação ou de incremento (AIx), um indicador da rigidez da parede arterial [224].

Este índice exprime em valor percentual o aumento da pressão sistólica em consequência do retorno precoce em sístole da onda reflectida na parede arterial, que é tanto maior quanto mais rígida for a parede. O AIx é calculado através da razão entre a pressão de amplificação (P2-P1) e a pressão de pulso (P2) medidos a partir da onda de pulso no período basal (Figura 8).

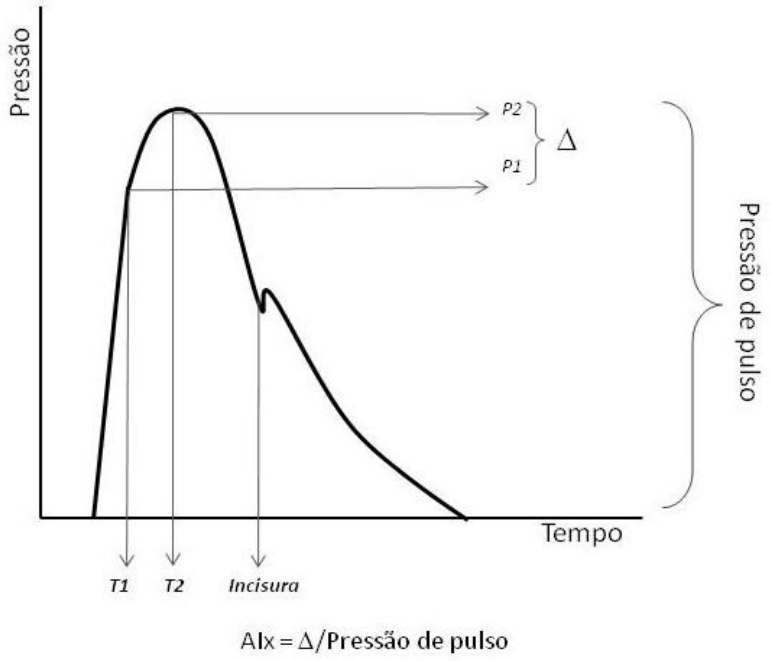

Figura 8 - AIx avaliado por PAT

O AIx é calculado dividindo o aumento de amplitude do 2º pico sistólico (P2-P1) pela pressão de pulso (P2)

Nas doenças reumáticas, o ecodoppler carotídeo e a TC coronária têm sido os métodos não invasivos mais utilizados na detecção de alterações vasculares estruturais subclínicas. A ecografia arterial com doppler permite avaliar alterações estruturais tais como a espessura da íntima-média, a presença de placas ateromatosas e o grau de estenose. O ecodoppler carotídeo com medição da espessura da íntima-média é largamente utilizado como indicador de doença

cardiovascular aterosclerótica pela boa correlação que tem com aterosclerose cerebral e coronária [225]. Na população geral, a espessura da íntima-média é possivelmente influenciada por determinantes genéticas [226], está relacionada com a presença de factores de risco CV [227] e é um bom preditor de EAM, particularmente nas mulheres [228].

A TC coronária permite detectar e quantificar o conteúdo de cálcio e o grau de estenoses coronárias, embora com discordância significativa relativamente à angiografia [229]. Um maior score de cálcio coronário acarreta um maior risco de eventos CV e tem um valor preditivo superior ao do score de Framingham em indivíduos assintomáticos [230]. Trata-se contudo de uma técnica com recurso a radiação, em que para a sua realização existem recomendações [231] e que ainda está longe de pertencer à rotina diagnóstica.

A ecografia endoluminal e a tomografia de coerência óptica das artérias coronárias são métodos importantes na caracterização da placa aterosclerótica [232] cuja performance nas doenças reumáticas nunca foi explorada. Também a utilidade clínica da ressonância magnética e da tomografia de emissão de positrões na avaliação da aterosclerose subclínica ainda é pouco clara, embora se trate de duas técnicas promissoras na avaliação das dimensões e caracterização da composição da placa aterosclerótica [233].

INFLAMAÇÃO SISTÉMICA E ATEROGÉNESE

A inflamação e a aterosclerose estão intimamente ligadas. Se por um lado a aterosclerose cursa com inflamação da parede vascular, por outro lado um processo inflamatório sistémico pode favorecer o aparecimento e a progressão das lesões vasculares.

Estudos epidemiológicos mostraram de forma inequívoca um risco vascular aumentado em indivíduos com níveis altos de citocinas pró-inflamatórias [234, 235] e de reagentes de fase aguda [236-239]. A elevação de parâmetros inflamatórios confere um risco idêntico ou superior àquele conferido pela hipercolesterolémia (Figura 8).

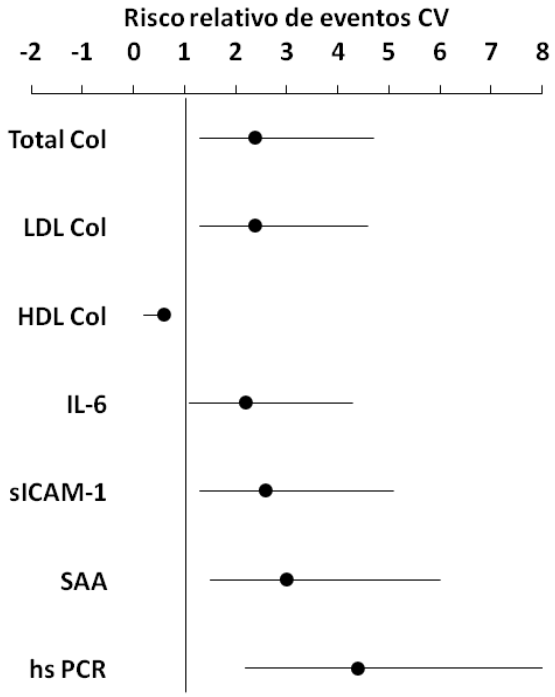

Figura 9 - Relação entre níveis séricos de marcadores de inflamação e o risco relativo de eventos CV

As mulheres pós menopáusicas com parâmetros inflamatórios elevados têm risco aumentado de eventos CV. Níveis altos de PCR de alta sensibilidade aumentam o risco CV mais de 4 vezes e são um preditor independente desse risco. O risco relativo apresentado no esquema refere-se aos valores do 4º quartil comparados com os do 1º quartil. Adaptado de Ridker P *et al* ref [240].

Adicionalmente, existe evidência de que diversos agentes infecciosos agravam o risco CV, provavelmente exercendo o seu efeito através de mecanismos inflamatórios [241, 242]. A favor da importância da inflamação sistémica estão ainda os resultados de ensaios clínicos demonstrando uma redução significativa do risco CV obtida com estatinas em indivíduos com elevação de parâmetros inflamatórios [243].

Níveis séricos aumentados de citocinas pró-inflamatórias favorecem a activação e disfunção das células endoteliais. O TNF, a IL-1 e o INFγ promovem a expressão de moléculas de adesão pelas células endoteliais e o recrutamento e activação de células inflamatórias, e podem ser responsáveis pelo despoletar da cascata da inflamação na parede arterial [146]. O fibrinogénio, frequentemente elevado nas doenças inflamatórias, ao ligar-se a receptores vasculares como o ICAM-1, funciona como elo fundamental para aumentar a adesão e migração transendotelial dos leucócitos [244].

A inflamação sistémica reflecte-se também nos factores de risco CV tradicionais, podendo por esta via proporcionar um contributo adicional para a aterogénese. São exemplos desta interacção o aumento da resistência à insulina e a modificação dos lípidos plasmáticos induzida por citocinas como o TNF ou a IL-6 [245-247]. A linfotoxina alfa é também uma citocina pró-inflamatória, estruturalmente semelhante ao TNF, com um papel chave na regulação do metabolismo lipídico [248]. O papel desta citocina na aterosclerose não está esclarecido, mas em modelos animais mostrou-se mais importante do que o TNF para a formação da placa aterosclerótica [249].

A relação entre inflamação e aterogénese é multifacetada. Do que foi exposto é evidente a existência de numerosos elos que estabelecem pontes entre as doenças reumáticas sistémicas, onde se incluem o LES e a AR, e a aterogénese. Foi essa a principal razão da escolha destas duas patologias como modelos de estudo da aterosclerose neste

projecto. O risco CV acrescido não é explicado cabalmente pela presença de factores de risco convencionais, cabendo à inflamação inerente à própria doença um contributo significativo. Apesar de serem ambas doenças inflamatórias, o excesso de eventos cardiovasculares é superior nos doentes com lúpus quando comparado com doentes com AR. A exploração dessas diferenças afigura-se assim como uma oportunidade para reconhecer factores relevantes para o risco CV. A identificação de particularidades inerentes a cada uma dessas doenças permitirá compreender melhor o risco CV associado e em última instância os mecanismos da aterogénese. A identificação de diferenças entre o LES e AR pode também abrir caminho à definição de estratégias preventivas mais apropriadas que reduzam a morbilidade e mortalidade CV nestas patologias.

OBJECTIVOS

O presente trabalho teve como objectivo principal compreender os mecanismos da aterosclerose prematura nas doenças reumáticas sistémicas, tendo como modelo o LES e a AR, ambas doenças inflamatórias crónicas mas com diferente risco CV.

Foram estudados doentes com LES, doentes com AR e controlos sem doença inflamatória crónica, todos do sexo feminino, com função renal normal e sem eventos CV prévios, com os seguintes objectivos específicos:

1. Estudar o impacto do LES e da AR na prevalência de factores de risco CV tradicionais e de outros factores predisponentes para doença aterosclerótica;

2. Avaliar a repercussão de mediadores da inflamação, de factores de risco CV tradicionais e de parâmetros hemorreológicos no desenvolvimento de aterosclerose subclínica no LES e na AR;

3. Avaliar a influência de polimorfismos genéticos para o risco CV;

4. Identificar marcadores precoces de lesão vascular nos doentes com LES e com AR e o efeito da inflamação e de factores de risco CV na activação e disfunção endoteliais.

RESULTADOS

De acordo com o art 8º do Decreto-Lei 388/70, art. 8º, os resultados apresentados e discutidos nesta tese foram publicados ou submetidos para publicação nas seguintes revistas científicas indexadas e revistas por peritos:

I. **Santos MJ**, Vinagre F, Canas da Silva J, Gil V, Fonseca JE. Cardiovascular risk profile in systemic lupus erythematosus and rheumatoid arthritis: a comparative study of female patients. Acta Reumatol Port. 2010 Jul-Sep;35(3):325-32.

II. **Santos MJ**, Vinagre F, Canas da Silva J, Gil V, Fonseca JE. Body composition phenotypes in systemic lupus erythematosus and rheumatoid arthritis: a comparative study of Caucasian female patients. Clin Exp Rheumatol. 2011 May-Jun;29(3):470-6. Epub 2011 Jun 29.

III. **Santos MJ**, Fernandes D, Caetano-Lopes J, Perpetuo IP, Vidal B, Canhao H, Fonseca JE. Lymphotoxin-α 252 A>G Polymorphism: A Link Between Disease Susceptibility and Dyslipidemia in Rheumatoid Arthritis? J Rheumatol. 2011 Jul;38(7):1244-9. Epub 2011 Apr 1

IV. **Santos MJ**, Pedro LM, Canhão H, Fernandes e Fernandes J, Canas da Silva J, Fonseca JE, Saldanha C. Hemorheological parameters are related to subclinical atherosclerosis in systemic lupus erythematosus and rheumatoid arthritis patients. Atherosclerosis. 2011;219:821–6. Epub 2011. Ago 26

V. **Santos MJ**, Carmona-Fernandes D, Canhão H, Canas da Silva J, Fonseca JE, Gil V
Different patterns of early vascular alterations in SLE and RA patients - a step towards understanding the associated cardiovascular risk.
Rheumatology (Submetido)

PARTE I

Cardiovascular risk profile in systemic lupus erythematosus and rheumatoid arthritis: a comparative study of female patients

CARDIOVASCULAR RISK PROFILE IN SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS: A COMPARATIVE STUDY OF FEMALE PATIENTS

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Abstract

Objective: Premature atherosclerosis is well-documented both in Systemic Lupus Erythematosus (SLE) and in Rheumatoid Arthritis (RA) patients, but cardiovascular (CV) risk is particularly high in lupus women. Although conventional CV risk factors do not fully explain the excessive risk in inflammatory diseases, they remain major contributors to atherosclerosis. The aim of the present study was to investigate whether CV risk factors are differentially associated with SLE and RA.

Methods: One hundred women with SLE, 98 with RA and 102 controls matched on age and without overt CV or renal disease were assessed for the presence of Framingham (hypertension, hypercholesterolemia, low HDL, diabetes, smoking) and other CV risks (atherogenic index of plasma (AIP), insulin resistance, obesity, central obesity, metabolic syndrome, uric acid, sedentarism, hypothyroidism and family history of premature CV disease).

Results: Modifiable CV risk factors are highly prevalent and occur more frequently in SLE and RA than in age-matched controls. Some differences in Framingham risk factors were found between SLE and RA, with hypertension being more common in young lupus women, hypercholesterolemia more frequent in RA and low HDL-C more frequent in SLE. However, the estimated 10-year Framingham CHD risk or the Reynolds Risk Score was comparable in both diseases. Although hypercholesterolemia was more frequent in RA, lupus women display a more atherogenic lipid profile, with significantly lower HDL-C levels (56.5±16 mg/dl versus 63.7±18; p=0.005), and more cases above the high risk cut-points for cholesterol/HDL-C (14% versus 4.1%; p=0.01) and for AIP (15% versus 6.1%; p=0.03). Also, uric acid levels are higher in SLE women (4.8±1.5 mg/dl) than in RA (4.1±1.1 mg/dl), p=0.001. On the other hand, insulin resistance is significantly higher in women with RA as compared with SLE (median HOMA-IR 3.5 [6.4]) versus 0.72 [2.5]; p<0.0001) and the difference remained significant after adjustment for BMI and corticosteroids.

Conclusions: Cardiovascular risk profile is distinct in SLE and RA women and the contribution of traditional CV risk factors to atherogenesis may be different in these two diseases. Prospective studies are necessary to understand how the control of modifiable risks can improve CV outcome in different inflammatory settings.

Introduction

The importance of premature atherosclerosis in Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA) is well established in population and cohort-based studies. Atherosclerotic complications account for the majority of late deaths in SLE[1] and represent the primary cause of death in half of the RA patients[2,3]. The incidence rates of myocardial infarction (MI) and angina in premenopausal lupus women is 50 times higher than in matched controls[4] and among RA women the observed incidence of MI is also greater than expected[5]. Furthermore, studies dealing with subclinical atherosclerosis showed the magnitude of this problem to be superior in SLE and RA as compared to the general population[6,7].

However, some differences in CV risk appear to exist between the two diseases. While the reported risk of ischemic heart disease is 5-8 folds higher in SLE patients[8,9], this increased risk is only 2-3 folds higher in RA patients[10,11]. In both diseases the rela-

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tive risk is more pronounced in youngest females[4,6,7].

Accelerated atherosclerosis in SLE and RA cannot be fully explained by traditional CV risk factors[8,11]. Nevertheless, conventional risk factors explain a substantial part of premature atherosclerosis[12,13] and, as many of them are modifiable, recommendations for their screening and control in inflammatory rheumatic disease have been developed[14,15].

The prevalence of hypertension, dyslipoproteinemia and sedentary lifestyle seems to be increased both in SLE and RA, though different studies provide a wide range of results [4,8,16,17,18]. However, there is limited information about the relative prevalence of CV risk factors in SLE and RA and to what extent this could account for the observed difference in CV events.

We examined the presence of classic Framingham and other conventional CV risk factors in SLE and RA women of similar age and without overt cardiovascular disease or renal function impairment to specifically address whether major differences exist between these two chronic inflammatory diseases of different pathogenesis. The Framingham 10-year risk of major heart events was estimated and, given the inflammatory setting of the study population, Reynolds Risk Score was also calculated. In addition, we assessed the distribution of CV risk factors in a control group to distinguish which factors are overrepresented in SLE and RA.

Material and methods

Patients

Adult women, fulfilling the American College of Rheumatology criteria for SLE or RA and attending the rheumatology clinic at Hospital Garcia de Orta in Almada, Portugal, on a regular basis, were recruited between January and December 2009. The control group consisted of women without chronic inflammatory disorders (patients with tendinitis or with low back pain) attending the same clinic. Exclusion criteria were: pregnancy, breastfeeding, prevalent myocardial infarction, angina pectoris, coronary revascularization, ischemic stroke and impaired renal function. In order to guarantee representativeness of different age groups, participants were enrolled in a consecutive way and allocated according to age frequency to obtain a final sample of 30% aged 18-39 years, 45% aged 40-59 years, and 25% aged ≥60 years. The study was approved by the local Ethics Committee and participants provided written informed consent.

Assessments

Participants underwent a structured interview, physical examination and laboratory evaluation. Age, ethnicity, menopausal status, number of years of education, smoking, physical activity, disease duration from the physician's diagnosis, current medication, co-morbidities and family history of CV events was assessed. Information on cumulative corticosteroid dose was obtained from review of patients' medical records. Current disease activity was evaluated using the SLE Disease Activity Index 2000 (SLEDAI2K)[19] and in RA patients 28 joints were examined for tenderness and swelling, and the disease activity score (DAS28) was calculated using erythrocyte sedimentation rate[20]. Damage was scored according to the Systemic Lupus International Collaborating Clinics/ACR Damage Index (SDI)[21] and RA functional status was evaluated using the Stanford Health Assessment Questionnaire Disability Index (HAQ)[22].

Standing height, weight, waist circumference and blood pressure were measured and body mass index (BMI) (Kg/m^2) calculated. A fasting blood sample was obtained for measurement of plasma glucose, insulin, total cholesterol, high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), triglycerides, uric acid and thyroid stimulating hormone (TSH). Insulin resistance was estimated by homeostasis model assessment (HOMA-IR) using the formula HOMA-IR= [fasting insulin (μU/ml) × fasting glucose (mmol/l)]/22.5. The ratio total cholesterol/HDL-C and the atherogenic index of plasma (AIP), defined as the logarithm of the ratio plasma triglycerides (mmol/l)/HDL (mmol/l)[23], were calculated. Cardiovascular risk factors were defined as follows: hypertension as a recorded blood pressure ≥140/90 mm/Hg or use of antihypertensive medication; dyslipidemia as a total cholesterol ≥200 mg/dl or LDL cholesterol ≥130 mg/dl or HDL cholesterol < 50 mg/dl or triglycerides ≥150 mg/dl or use of lipid-lowering agents; diabetes as a fasting glucose level ≥126 mg/dl, a self-reported physician diagnosis or pharmacologic treatment; insulin resistance was defined by an HOMA-IR in the top quartile of a non-diabetic population (>2.114)[24], impaired fasting glucose (≥110mg/dl) or diabetes; obesity was considered if

BMI ≥30 Kg/m2; central obesity if the waist circumference was above the IDF population recommended cut-points[25]; current smoker if the participant smoked ≥1 cigarettes per day during the last month; sedentary lifestyle if the amount of self-reported weekly exercise during the last 12 months was <3 times or <30 min per session; family history of premature CV events was defined as myocardial infarction or ischemic stroke occurred in a first-degree relative before the age of 55 years in males or before the age of 65 years in females. Metabolic syndrome was diagnosed according to the joint definition of the International Diabetes Federation, National Heart Lung and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity and using waist circumference ≥80 cm as the threshold for abdominal obesity[26].

For estimation of absolute 10-year risk for major coronary heart disease (CHD) events we utilized the predictive model based on the Framingham risk score algorithm developed by Wilson *et al*[27]. This algorithm assesses the probability of having a MI or cardiac death during the next 10 years on the basis of the risk factor profiles and was developed for people 30 to 74 years old. The model includes gender, age, diabetes, smoking status, blood pressure, total cholesterol and HDL cholesterol as risk predictors. The Reynolds Risk Score predicts global cardiovascular risk (MI, coronary revascularization, ischemic stroke and cardiovascular mortality) and incorporates C-reactive protein levels (CRP) and family history of premature CV disease[28]. The 10-year risks were categorized into low (predicted risk <10%), intermediate (predicted risk 10-20%) and high (predicted risk >20%)[29].

Statistical analysis

Calculations were performed using SPSS 17.0 software for Windows and a 2-tailed p value <0.05 was selected as significant. Continuous variables are presented as means ± standard deviations or medians and inter-quartile ranges depending on whether the data were normally distributed or not. Categorical variables are reported as proportions. Comparisons between SLE and RA groups were made using chi-square or Fisher's exact test and Student t-tests for independent samples. The Mann Whitney U tests were used to compare variables that were highly skewed. Intergroup differences in CV risk factors were also calculated between each age stratum: 18-39 years, 40-59 years and ≥ 60 years. We also compared the disease group (SLE plus RA) with the control group using the same tests.

To evaluate the independence of the association between the diagnosis and CV risk factors we used binary logistic regression analysis. HOMA-IR, AIP and cholesterol/HDL-C ratio were dichotomized below and above high risk cutoffs. Multiple linear regression analysis was used to assess the independent relationship between diagnosis and lipid levels.

Results

Demographic and disease characteristics

We studied 300 women, 100 with SLE, 98 with RA and 102 controls, with a similar mean age, but a higher proportion of non-Caucasians among patients (14% of SLE and 11.2% of RA versus 2.9% of controls). The educational level and the proportion of postmenopausal women were comparable in the three groups (Table I). The median duration since SLE diagnosis was 6.6 (range 0.5 - 34) years. Arthritis had occurred in 78%, renal disease in 28%, serositis in 26%, hemolytic anemia in 15% and lupus psychosis in 5% of SLE cases. All SLE patients were ANA positive, 80% were anti-dsDNA positive and 37% tested positive either for anti-cardiolipin, lupus anticoagulant or both. At the time of the evaluation 83% had low disease activity (SLEDAI2K <6) and 42% presented some irreversible damage (SDI≥1). Sixty patients were taking steroids in a median daily dose of 7.5 mg (range 1.25 to 60 mg) of prednisolone equivalent. The majority (73%) was currently on antimalarials and 24% on immunossupressants.

The median duration of RA was 7.6 (range 0.5 to 30) years, 59.2% had erosive disease and 88.8% tested positive either for rheumatoid factor (RF), anti-CCP antibodies or both. The mean disease activity score (DAS28) was 4.24 and the mean HAQ 1.15. Fifty two (53.1%) patients were taking corticosteroids in a median dose of 5 mg/day (range 2.5 to 15 mg), 89 (91%) were currently receiving synthetic DMARDs, the most commonly used was methotrexate in 81 cases, and 39 (40%) were on biologics.

Framingham CHD risk factors and predicted 10-year risk

The presence of Framingham CHD risk factors, i.e. hypertension, hypercholesterolemia, low-HDL,

Table I. Demographic and clinical characteristics of study subjects

Characteristic	SLE (n = 100)	RA (n = 98)	Controls (n = 102)
Age, years	46.6 ± 13.4	49.2 ± 13.7	47.7±13.4
Caucasian, (%)	86 (86)	87 (88.8)	99 (97.1)
Education, years	8.5 [8]	9 [8]	9 [8]
Postmenopausal, (%)	50 (50)	54 (55.7)	55 (53.9)
Disease duration, years	6.6 [6.8]	7.6 [9.1]	–
SLEDAI2K	2 [4]	–	–
DAS 28	4.24 ± 1.28	–	–
SDI	0 [1]	–	–
HAQ	1.15 ± 0.7	–	–
NSAIDs, (%)	28 (28)	62 (63.3)	23 (22.5)
Steroids, (%)	60 (60)	52 (53.1)	–
Current steroid dose, mg	7.5 [12]	5 [0]	–
Cumulative steroid dose, g	7.7 [18]	6.4 [15]	–
Methotrexate therapy, (%)	9 (9)	81 (82.7)	–
Antimalarial therapy, (%)	73 (73)	19 (19.4)	–
TNF blocker therapy, (%)	–	39 (40)	–
Immunosuppressive therapy, (%)	24 (24)	–	–

Continuous variables are presented as means ± SD or median [IQR] according to the distribution and categorical variables as absolute values and proportions (%)

diabetes and smoking, occurred very often in the studied population: 83% of SLE, 79.6% of RA and 81% of control women presented at least on risk factor. The mean number of Framingham CHD risk factors was similar in SLE, RA and controls (1.56±0.9; 1.57±0.9 and 1.51±1.1, respectively). Patients were significantly more likely to have hypertension (OR=1.97, 95%CI 1.19-3.24, p=0.008); otherwise the distribution of CHD risk factors was similar in the 3 groups. The mean Framingham 10-year risk of major CHD events was 4.4% for SLE, 4.9% for RA and 4.5% for controls. Similarly, Reynolds Risk Score did not show significant differences among the groups: SLE 2.5%; RA 2.8%; controls 2.2%.

Hypertension was significantly more prevalent in SLE women aged 18-39 years (34.3%) than in RA women within the same age range (11%; p=0.04). The mean values of systolic and diastolic blood pressure in SLE and RA were 128±21/76±11 mmHg and 127±21/75±12 mmHg, respectively, but more women in the SLE group received anti-hypertensive treatment (45% vs 32.6%; p=0.05). Differences between SLE and RA groups could be detected in the prevalence of hypercholesterolemia and low-HDL, but again lupus patients were found to receive lipid-lowering treatment more frequently than RA (39% vs 20.4%; p=0.004).

Lipid levels and atherogenic index of plasma

Fasting lipid levels were measured in all participants and mean values are shown in Table III. To determine the independent association of SLE and RA diagnosis with lipid levels we used multiple regression analysis adjusted for the identified potential confounders (lipid lowering therapy and current corticosteroid dose). SLE diagnosis was significantly associated with lower levels of total cholesterol as well as HDL-C and LDL-C. However, when we calculated the balance between risk and protective lipid fractions, lupus women were more likely to have cholesterol/HDL ratio (OR=3.92, 95%CI 1.24 to 12.48, p=0.02) and AIP values (OR=2.77, 95%CI 1.03-7.49, p=0.04) above the high risk cut-points.

Distribution of other CV risk factors

Several CV risk factors have been described beyond the classic Framingham ones. We evaluated the presence of insulin resistance, obesity, abdominal obesity, metabolic syndrome, hypothyroidism, uric acid levels and family history of premature CV disease. Table IV shows the presence of these risk factors in SLE, RA and control women.

HOMA index and uric acid levels are significantly higher in patients than in controls, as well as the proportion of insulin resistance, central obesity,

Table II. Distribution of traditional Framingham CHD risk factors in women with SLE, RA and non-inflammatory controls

	SLE (n =100)	RA (n = 98)	p-value	Controls (n=102)
Hypertension, (%)	53 (53)	43 (43.9)	ns	33 (32.4)*
18-39y	12 (34.3)	3 (11.1)	0.04	2 (6.5)†
40-59y	25 (58.1)	16 (36.4)	ns	16 (33.3)
≥60y	16 (72.7)	24 (88.9)	ns	15 (65.2)
Hypercholesterolemia, (%)	37 (37)	53 (54)	0.01	57 (55.9)
18-39y	12 (34.3)	9 (33.3)	ns	13 (41.9)
40-59y	11 (25.6)	26 (60.5)	0.001	27 (56.3)
≥60y	7 (37.2)	18 (66.7)	ns	17 (73.9)
Low HDL-C, (%)	39 (39)	20 (20.4)	0.005	24 (23.8)
18-39y	15 (45.5)	5 (18.5)	0.02	7 (22.3)
40-59y	15 (37.5)	9 (20.9)	ns	11 (23.4)
≥60y	9 (37.4)	6 (22.2)	ns	6 (26.1)
Diabetes, (%)	8 (8)	7 (7.1)	ns	7 (6.9)
Current smoker, (%)	15 (15)	17 (17.3)	ns	19 (18.6)
18-39y	7 (20)	6 (22)	ns	9 (29)
40-59y	8 (18.6)	11 (25)	ns	10 (20.8)
≥60y	0	0	ns	0
Framingham CHD risk ≥ 10%, (%)	11/88 (12.5)	16/91(17.6)	ns	15/91 (16.5)

*significant difference between patients and controls p=0.008; †p=0.04

Table III. Lipid levels and atherogenic index of plasma

	SLE (n=100)	RA (n=98)	Adjusted p*	Controls (n=102)
Total cholesterol, mg/dl	191.6 ± 43.5	203.3 ± 33.8	0.001	206.9 ± 33.8
LDL-C, mg/dl	114.7 ± 37	122.8 ± 28	0.01	127.9 ± 29
HDL-C, mg/dl	56.5 ± 16	63.7 ± 18	0.006	62.2 ± 15
Triglycerides, mg/dl	125.3 ± 78	103.9 ± 44	ns	98 ± 41
Cholesterol/HDL-C >5, (%)	14 (14)	4 (4.1)	0.01	9 (8.8)
AIP >0.21, (%)	15 (15)	6 (6.1)	0.03	6 (5.9)

The proportion of patients receiving lipid lowering medication was: SLE-39%; RA-20.4%; controls- 24.5% (p=0.009)
AIP– atherogenic index of plasma
*adjusted for use of lipid lowering therapies, and current corticosteroid dose

metabolic syndrome and hypothyroidism. More RA than SLE women are insulin resistant (OR=3.33, 95%CI 1.75-6.36, p=<0.0001) and the association between insulin resistance and RA diagnosis remained significant after adjustment for BMI and current corticosteroid dose (p=0.02).

We found uric acid concentration to be higher in lupus women, even in the absence of renal impairment or history of gout. The relationship between uric acid and SLE diagnosis remained significant irrespective of the use of anti-hypertensive medication (p=0.004). Additionally, more RA patients had a positive family history of premature cardiovascular events.

Discussion

In the present study we found CV risk factors to be very common in SLE and RA women and that some

Table IV. Distribution of other conventional cardiovascular risk factors

	SLE (n =100)	RA (n = 98)	p-value	Controls (n=102)
HOMA-IR	0.72 [2.5]	3.5 [6.4]	0.0001	0.46 [0.9]*
Insulin resistant, n (%)	35 (35)	61 (62.2)	0.0001	20 (19.6)*
Obesity, n (%)	28 (28)	31 (32)	ns	28 (27.5)
18-39y	8 (22.9)	5 (19.2)	ns	3 (9.3)
40-59y	15 (27.3)	12 (34.9)	ns	14 (29.2)
≥ ≥60y	5 (22.7)	14 (51.4)	ns	11 (47.8)
Central obesity, n (%)	72 (72)	78 (79.6)	ns	64 (62.3)**
18-39y	20 (57.1)	20 (66.3)	ns	11 (35.5)**
40-59y	35 (81.4)	33 (75.6)	ns	34 (70.8)
≥60y	17 (77.3)	25 (95.8)	ns	19 (82.6)
Sedentary lifestyle, n (%)	84 (84)	87 (88.7)	ns	83 (82.2)
18-39y	28 (80)	20 (74.1)	ns	24 (80)
40-59y	35 (81.4)	42 (95.5)	ns	42 (87.5)
≥60y	21 (95.5)	25 (92.6)	ns	17 (73.9)
Metabolic Syndrome	27 (27)	25 (25.5)	ns	16 (15.7)†
18-39y	5 (14.3)	2 (7.7)	ns	0 (0)†
40-59y	12 (27.9)	12 (27.3)	ns	7 (14.6)
≥60y	10 (45.5)	10 (37)	ns	9 (39.1)
Hypothyroidism (%)	7 (7)	11 (11.2)	ns	3 (2.9)†
Uric acid, mg/dl	4.8 ± 1.5	4.1 ± 1.1	0.001	3.8 ± 1.0*
Family history of premature CV disease, n (%)	2 (2)	14 (14.3)	0.002	14 (13.7)

*significant difference between patients and controls p=0.0001; ** p=0.02; †p=0.04

differences could be detected between these two inflammatory diseases and the control population. Metabolic syndrome, as well as some features of this syndrome, such as hypertension, insulin resistance, and central obesity, are overrepresented among SLE and RA patients. Decreased thyroid function was also found more frequently in both patient groups.

We could also depict differences in the prevalence of CV risk factors between SLE and RA women. With regard to Framingham risk factors, hypertension is more common in young lupus women and the alterations in lipid profile are distinct in SLE and RA patients. Despite lower total cholesterol, lupus women present a more atherogenic lipid profile characterized by lower HDL-C and a more harmful balance between risk and protective lipid fractions. Furthermore, the association of atherogenic lipid profile with SLE diagnosis persists, irrespective of the use of lipid-lowering agents or corticosteroids. Indeed, systemic inflammation may induce alterations in lipoprotein profile and in SLE patients it was shown that both disease activity and medication (corticosteroids and lipid-lowering agents) contribute to the changes in lipids that occur over time[30]. Active RA is also associated with a more atherogenic lipid profile and these changes have been documented even years before RA was diagnosed[31]. Furthermore, not only the levels of atheroprotective lipids are decreased, but also their function may be impaired in inflammatory rheumatic diseases[32]. Thus, identification of a more atherogenic lipid profile in lupus women, despite low disease activity and greater use of lipid-lowering agents is of interest because this suggests that the mechanisms underlying dyslipoproteinemia may differ in different inflammatory conditions.

In the general population, increased uric acid levels are an independent marker of cardiovascular disease[33] and arterial stiffness may represent the link between hyperuricemia and atherosclerosis[34]. We found higher uric acid levels in lupus women, despite normal creatinine levels and the absence

of gout. This increase was independent of the use of anti-hypertensive medication. We cannot rule out a subclinical renal impairment underlying the increased uric acid. Nevertheless, elevated uric acid may represent an additional risk for CV disease in lupus patients.

Insulin resistance, which is fundamental to the metabolic syndrome, was associated with symptomatic cardiovascular disease in the general population[35]. We found HOMA-IR to be significantly increased in RA as compared with SLE and this increase to be independent of BMI or corticosteroids. A previous study also demonstrated higher HOMA index in RA than in SLE and the association of insulin resistance with inflammation and coronary atherosclerosis[36]. This may be an important target to reduce CV risk in this patient population.

Hypothyroidism is associated with a wide range of metabolic changes, including increased BMI and adverse lipoprotein profile. There is also a relationship between hypothyroidism, vascular dysfunction and accelerated atherosclerosis[37]. Although hypothyroidism was more frequent in patients than in controls, its prevalence was similar in SLE and RA women.

A comprehensive identification of cardiovascular risk profile of SLE and RA is an opportunity to improve health management of these patients. As most of the identified risk factors are susceptible of intervention and modification, future research is crucial in order to establish to what extent the control of modifiable risk factors can improve cardiovascular outcome of these patients.

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PARTE II

Body composition phenotypes in systemic lupus erythematosus and rheumatoid arthritis: a comparative study of Caucasian female patients.

Body composition phenotypes in systemic lupus erythematosus and rheumatoid arthritis: a comparative study of Caucasian female patients

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Abstract

Objective

The amount and distribution of fat and lean mass have important implications for health and systemic inflammation may represent a risk for altered body composition. The aim of this study was to analyse whether changes in body composition are similarly associated with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), two inflammatory conditions of different pathogenesis.

Methods

Body mass index (BMI), waist circumference, fat mass (FM) and fat-free mass (FFM) were measured in 92 women with SLE, 89 with RA and 107 controls. Results were compared among the 3 groups and correlations of FM percentage were explored within SLE and RA.

Results

Abnormal body composition was more frequent in women with SLE and RA than in non-inflammatory controls, despite having a similar BMI. RA diagnosis was significantly associated with overfat (OR=2.782, 95%CI 1.470–5.264; p=0.002) and central obesity (OR=2.998, 95%CI 1.016–8.841; p=0.04), while sarcopenia was more common among SLE (OR=3.003; 95%CI 1.178–7.676; p=0.01). Sarcopenic obesity, i.e. the coexistence of overfat with sarcopenia, was present in 6.5% of SLE and 5.6% of RA women, but no controls. Independent correlations of FM percentage in women with SLE included smoking, disease activity and CRP. In RA, education, disease activity and cumulative corticosteroid dose were identified as independent predictors of FM percentage.

Conclusion

Women with SLE or RA diagnosis are more likely to have abnormal body composition phenotype, with some differences existing between these two conditions. Changes in body composition are partly explained by the inflammatory burden of disease and its treatment.

Key words

obesity, sarcopenia, systemic lupus erythematosus, rheumatoid arthritis, inflammation

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Introduction

The amount and distribution of body fat and lean mass have important implications for health. A very low body mass index (BMI) as well as obesity are associated with reduced life expectancy, increased morbidity and poor quality of life (1-4). Not only the amount of fat, but also the distribution of adipose tissue may have detrimental consequences on health. In fact, a disproportionate accumulation of adipose tissue in the abdominal region, which is a key feature of metabolic syndrome, is associated with a prothrombotic and proinflammatory state and confers a higher risk of developing cardiovascular (CV) diseases (5, 6). Additionally, by secreting adipokines, adipose tissue actively participates in the inflammatory process and higher concentrations of inflammatory markers are present in overweight and obese people, as well as in association with metabolic syndrome (3, 7).

Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are multisystemic rheumatic diseases that affect joints and muscles, causing pain, disability and increased risk for physical inactivity (8). Patients often require prolonged therapy with corticosteroids which contributes to weight gain (9). Also, the systemic inflammatory nature of these diseases may, *per se*, represent an additional risk for altered body composition. In fact, in RA both cachexia and excessive fat mass have been identified more frequently than in the general population (10, 11). In the same way, abnormal body composition phenotypes may also be overrepresented among patients with SLE (12, 13), although information regarding this patient group is limited. Moreover, metabolic syndrome was found more commonly in SLE and RA than in controls matched for age and sex (14, 15). Changes in body composition may therefore represent an additional risk for cardiovascular diseases in SLE and RA.

The present work was undertaken to examine whether changes in body composition are similarly associated with SLE and RA, two chronic inflammatory conditions of different pathogenesis, and to what extent the disease characteristics and pharmacotherapy affect the percentage of body fat mass.

Material and methods

Study population

Adult Caucasian women, fulfilling the American College of Rheumatology (ACR) criteria for SLE or for RA and attending the rheumatology clinic at Hospital Garcia de Orta in Almada, Portugal, on a regular basis, were eligible for the study. The control group consisted of women without chronic inflammatory disorders (patients with tendinitis or with low back pain) attending the same clinic. Exclusion criteria were pregnancy, breastfeeding, ethnicity other than Caucasian, functional class IV as defined by the ACR classification of functional status, ischemic heart disease, and impaired renal function (serum creatinine >1.6 mg/dl). The study was approved by the local Ethics Committee and participants provided written informed consent.

Between January and December 2009 a total of 305 women were recruited. Five declined participation and 12 were excluded. The reasons for exclusion were heart disease in 9 cases and renal insufficiency in 3. The final study population comprised 288 women: 92 with SLE, 89 with RA and 107 controls. Demographic and disease characteristics are summarised in Table I.

Participants underwent a structured interview and physical examination performed by the same trained investigators.

Anthropometric and body composition measurements

All assessments were carried out during the morning of the study visit day with participants in the fasting state in order to minimise circadian variations. Standing height (in cm), and body weight (in kg) were measured with patients wearing light clothes and without shoes, and body mass index (BMI) (kg/m^2) was calculated. Waist circumference was measured at the end of the normal expiration with a tape placed horizontally midway between the lower edge of the rib cage and the iliac crest, and the mean of two measurements was taken in account. Total fat mass

Table I. Demographic and clinical characteristics of studied women.

	SLE (n=92)	RA (n=89)	Controls (n=107)
Demographics and lifestyle			
Age, years	46.8 ± 14.1	49.8 ± 13.8	47.5 ± 13.1
Menopause (%)	46 (50 %)	51 (57.3 %)	58 (54.2%)
Education, years	9.3 ± 5.1	8.8 ± 4.8	8.4 ± 5.3
Current smoker (%)	13 (14.1%)	16 (16.9%)	23 (21.5%)
Physically active (%)	18 (19.6 %)	10 (11.2%)	20 (18.7%)
Disease characteristics			
Disease duration, years	8.5 ± 6.9	9.7 ± 7.1	NA
SLEDAI 2K	2 [4]	–	NA
DAS28	–	4.24 ± 1.3	NA
SDI	0 [1]	–	NA
HAQ	–	1.16 ± 0.73	NA
Corticosteroids			
Current use (%)	52 (56.5%)	46 (51.7%)	NA
Current daily dose, mg	5 [8.8]	2.5 [5]	NA
Cumulative dose, g	7.3 [20.7]	7.3 [15]	NA

Data is presented as mean ± standard deviation for normally distributed, median and interquartile range [IQR] for non-normally distributed continuous variables and proportions (%) for categorical variables.
SLEDAI2K: Systemic lupus erythematosus disease activity index 2000; DAS28: Disease activity score; SDI: Systemic lupus international collaborating clinics/ACR damage index; HAQ: Health assessment questionnaire.

(in kg), percentage of body fat, and fat-free mass (in kg) were obtained by bioelectrical impedance analysis (BIA), using an Omron HBF-510 Full Body Composition analyser. BIA is a simple method for routine clinical use that correlates well with hydrodensitometry body fat analysis and with the "gold-standard" dual-energy x-ray absorptiometry (DXA) measurement (11). Fat mass index (FMI) was calculated by dividing body fat mass by the square of the height (kg/m^2) and fat-free mass index (FFMI) by dividing fat-free mass by the square of the height (kg/m^2).

Clinical assessment

Socio-demographic data (age, menopausal status, education), health habits (smoking status, physical activity), disease duration, current and past medications and co-morbidities were assessed by patient self-report and information was completed by review of medical records.

SLE disease activity was evaluated using the SLEDAI2K (16), a measure of ongoing disease activity that ranges from 0 to 105. Damage was scored according to the Systemic Lupus International Collaborating Clinics/ACR Damage Index (SDI) (17) from 0 to 46. In RA patients 28 joints were examined for tenderness and swelling, and the disease activity score (DAS28) was calculated using ESR. Disease activity was classified as low (DAS28 <3.2), medium (DAS28 3.2–5.1) or high (DAS28 >5.1) (18). Functional status (disability) was evaluated using the Stanford Health Assessment Questionnaire Disability Index (HAQ) (19), a self-administered questionnaire that gives a score range from 0 to 3, with higher scores indicating lower functional capacity. SDI and HAQ were used as surrogate markers of cumulative disease severity. Blood and urine samples were collected and the required tests performed in order to calculate SLE and RA disease activity (complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), creatine kinase, anti-DNA antibody titer, C3 and C4 level and urinalysis).

Definitions

Participants were classified as physically active if the amount of self-reported weekly aerobic exercise during the last 12 months was ≥3 times and ≥30 min per session. Individuals with BMI <18.5 kg/m^2 were considered underweight, between 18.5 and 24.9 normal, between 25 and 29.9 overweight and with BMI values ≥30 obese. Abdominal obesity was defined in agreement with WHO (20) and IDF (21) population recommended cut-points, which corresponds to a waist circumference ≥80 cm in Caucasian women. Overfat was defined according to total body FM percentage assessed by BIA. Women were considered overfat if their percentage of body fat was superior to 40.01% (22). The cutoff to define sarcopenia was a FFMI ≤2 SD below the mean of a reference Caucasian population (23). On the basis of FM percentage and FFMI participants were categorised into purely obese (overfat), purely sarcopenic, sarcopenic-obese and normal body composition.

Statistical analysis

Anthropometric and body composition parameters of SLE, RA and control women were compared using one-way analysis of variance for normally distributed continuous variables, with post hoc Bonferroni correction. To compare categorical variables Pearson χ^2 test or Fisher's exact test were used.

The independent association between the inflammatory diseases and body composition phenotype was assessed by multiple logistic regression. The dependent variable "body composition" was dichotomised into normal body composition phenotype or altered body composition (purely obese, purely sarcopenic and sarcopenic obese) and the diagnosis, as well as potential confounders (age, BMI, current and cumulative corticosteroid dose), were the covariates.

Subsequently, the impact of clinical features and treatment on fat mass percentage was investigated separately among patients with SLE and those with RA by using linear logistic regression. Variables related in univariate analyses to FM percentage at a *p*-value <0.20 were considered possible predictors and included in the logistic model. Before performing regression analysis FM percentage was logarithmically transformed to obtain normality, as well as disease activity, SDI, corticosteroid dose, and CRP to ensure the normality of the residuals in multiple linear regression. Statistical calculations were performed using SPSS 17.0

software and a 2-tailed *p*-value <0.05 was selected as significant.

Results

Patients' characteristics

Patients and controls had similar socio-demographic and lifestyle characteristics, including age (*p*=0.31) and percentage of post-menopausal women (*p*=0.61). SLE and RA women did not differ significantly with regard to disease duration or current corticosteroid use. Overall, 52 (56.5%) women with SLE and 46 (51.7%) with RA were on steroids at the time of evaluation. Among those taking steroids, the median daily dose was higher in SLE, but the cumulative corticosteroid dose was comparable in the 2 groups. The proportion of lupus women on antimalarials and on immunossupressants was 73% and 33.6%, respectively. Eighty two RA women were treated with synthetic disease-modifying anti-rheumatic drugs (DMARDs), of which 92.7% were on methotrexate (MTX); 39.3% received biologics. The majority of SLE patients (80.4%) had low disease activity (SLEDAI<6) with a median SLEDAI value of 2 [IQR 0 to 4]; 25.3% of RA patients had low, 44.2% moderate and 30.5% high disease activity and the mean DAS28 score was 4.24±1.3. Some irreversible damage was present in 40.2% of SLE patients and the median SDI value was 0 [IQR 0 to 1]. The mean HAQ score of RA patients was 1.16 ±0.73.

Anthropometric and body composition measurements

Table II shows anthropometric and body composition measurements. Patients and controls had comparable mean BMI. Patients with BMI<25 kg/m^2 were younger (*p*<0.001), pre menopause (*p*=0.006), current smokers (*p*=0.04), had higher education level (*p*<0.0001) and more active disease (*p*=0.03), compared with those with BMI≥25 kg/m^2. No differences were found regarding physical active persons, disease duration, disease severity, and corticosteroid use or dose.

Body fat mass (FM) was significantly higher in women with RA than in non-inflammatory controls across all BMI categories. Within the normal BMI range, SLE women also presented higher amount of FM. RA diagnosis was associated with greater odds of overfat (OR=2.782, 95%CI 1.470 to 5.264, *p*=0.002). Low FM was exceptional; only 2 individuals in each inflammatory group and no controls had less than 20% of body fat. The mean value of FFM was similar across the 3 groups. However, significantly more SLE patients were sarcopenic (OR=3.003; 95%CI 1.178 to 7.676; *p*=0.01). Moreover, 6.5% of SLE patients, 5.6% of RA patients but no controls could be classified as sarcopenic obese (*p*=0.03).

Compared with the control group, both SLE and RA women were less likely to have normal body composition. The adjusted OR for altered body composition was 2.581 (95% CI 1.234–5.396,

Table II. Anthropometric and body composition characteristics of women with SLE, RA and control subjects.

	SLE (n=92)	RA (n=89)	Controls (n=107)	*p*-value
BMI, kg/m^2	27.0±4.9	27.6±5.0	26.7 ± 4.7	NS
FM, kg	24.2±8.6	26.5±8.8*	22.5 ± 7.8	0.006
FFM, kg	42.8±7.0	42.6±6.0	42.8 ± 6.6	NS
FM (kg) per BMI category:				
Nomal	17.1±4.2*	18.0±5.5*	14.5 ± 4.3	0.04
Overweight	24.7±3.8	25.9±4.0*	23.6 ± 3.3	0.05
Obese	34.2±6.6	35.6±6.7*	31.9 ± 4.5	0.04
Body composition:				
Purely overfat	19 (20.7%)	31 (34.8%)*	20 (18.7%)	0.005
Purely sarcopenic	10 (10.9%)*	4 (4.5%)	7 (6.5%)	0.04
Sarcopenic obese	6 (6.5%)*	5 (5.6%)*	0 (0%)	0.03
Healthy composition	56 (60.9%)*	49 (55.1%)*	80 (74.8%)	0.01
Central obesity, %	65 (70.7%)	79 (77.5%)*	67 (62.6%)	0.04

Variables are presented as mean ± SD or proportions (%).The three groups were compared using one-way analysis of variance, χ^2 or Fisher's exact test with post-hoc Bonferroni correction for multiple comparisons. BMI: body mass index; FM: fat mass; FFM: free-fat mass.
*Significant differences between groups are as follows: FM - RA *vs*. controls *p=0.004*; Fat mass within normal BMI range - SLE *vs*. controls *p=0.04*, RA *vs*. controls *p=0.03*; Fat mass within overweight BMI range - RA *vs*. controls *p=0.04*; Fat mass within BMI obese range - RA *vs*. controls *p=0.02*; Body composition: Purely overfat - RA *vs*. controls *p=0.001*; Purely sarcopenic - SLE *vs*. controls *p=0.01*; Sarcopenic obese - SLE *vs*. controls *p=0.009*; RA *vs*. controls *p=0.01*; Healthy body composition - SLE *vs*. controls *p=0.04*; RA *vs*. controls *p=0.004;* Central obesity - RA *vs*. controls *p=0.02*

Table III. Predictors of FM percentage in women with SLE.

	Univariate regression analysis		Multiple regression analysis†	
Explanatory variables	β coefficient*	*p*-value	β coefficient*	*p*-value
Education, years	-0.022 (-0.031 to -0.012)	<0.0001	-0.003 (-0.013 to 0.007)	0.58
Smoking, Y/N	-0.221 (-.355 to -0.089)	0.001	-0.13 (-0.243 to -0.016)	0.02
Physically active, Y/N	-0.086 (-0.207 to 0.036)	0.16	-0.004 (-0.112 to 0.104)	0.93
Disease duration, years	0.058 (-0.004 to 0.119)	0.07	0.032 (-0.028 to 0.092)	0.29
Log (SLEDAI2K)	-0.053 (-0.106 to 0.000)	0.05	-0.049 (-0.096 to -0.003)	0.03
Log (SDI)	0.218 (0.006 to 0.175)	0.03	-0.018 (-0.103 to 0.068)	0.69
Immunosuppressant use, Y/N	0.020 (-0.084 to 0.124)	0.70	–	–
Log (Current corticosteroid dose)	0.005 (-0.036 to 0.045)	0.82	–	–
Log (Cumulative corticosteroid dose)	0.002 (-0.013 to 0.017)	0.79	–	–
Log (CRP)	0.100 (0.01 to 0.185)	0.02	0.088 (0.017 to 0.16)	0.01

FM: Fat mass; SLEDAI2K: Systemic lupus erythematosus disease activity index 2000; SDI: Systemic lupus international collaborating clinics /ACR damage index; CRP: C-reactive protein.
*Unstandardised coefficients; †Multiple linear regression analysis was adjusted for age. The total explained variance of the model is R^2=0.448.

p=0.01) in SLE and 2.592 (95% CI1.246–5.392, p=0.01) in RA.

The prevalence of central obesity differed significantly from controls only in RA women. After adjustment for covariates, the risk of central obesity was almost 3 times higher in RA (OR=2.998, 95%CI 1.016–8.841; p=0.04).

The relationships between clinical features and percentage of FM in SLE women are presented in Table III. In univariate analysis there was a significant association of FM percentage with education, smoking, disease activity, damage and CRP. When possible predictors were entered in multiple linear regression analysis, smoking, disease activity and CRP were identified as independent predictors of FM percentage in lupus women.

Table IV shows the relationships between clinical features and percentage of fat mass in RA women. A strong association of education, HAQ and cumulative corticosteroid dose with FM was observed. However, when clinically important and possible predictors were adjusted for, education, disease activity and cumulative corticosteroid dose were identified as independent predictors of FM percentage in RA.

Table IV. Predictors of FM percentage in women with RA.

	Univariate regression analysis		Multiple regression analysis†	
Explanatory variables	β coefficient*	p-value	β coefficient*	p-value
Education, years	-0.023 (-0.032 to -0.014)	<0.0001	-0.011 (-0.021 to 0.000)	0.04
Smoking, Y/N	-0.126 (-0.236 to 0.010)	0.06	0.004 (-0.127 to 0.118)	0.94
Physically active, Y/N	-0.029 (-0.193 to 0.136)	0.73	–	–
Disease duration, years	0.07 (-0.002 to 0.143)	0.06	-0.004 (-0.010 to 0.003)	0.27
DAS28	-0.022 (-0.062 to 0.018)	0.13	-0.038 (-0.076 to -0.001)	0.04
HAQ	0.089 (0.015 to 0.158)	0.01	0.019 (-0.051 to 0.089)	0.59
DMARD use, Y/N	-0.151 (-0.342 to 0.040)	0.12	-0.047 (-0.206 to 0.112)	0.55
TNF inhibitors, Y/N	0.096 (-0.007 to 0.198)	0.07	0.018 (-0.076 to 0.111)	0.70
Log (Current corticosteroid dose)	0.037 (-0.017 to 0.09)	0.17	-0.005 (-0.053 to 0.055)	0.95
Log (Cumulative corticosteroid dose)	0.047 (0.020 to 0.075)	0.001	0.043 (0.014 to 0.073)	0.005
Log (CRP)	-0.05 (-0.155 to 0.055)	0.34	–	–

FM: Fat mass; DAS28: Disease activity score; HAQ: Health assessment questionnaire; DMARD: Disease-modifying anti-rheumatic drug; TNF: tumour necrosis factor
*Unstandardised coefficients. †Multiple linear regression analysis was adjusted for age. The total explained variance of the model is R^2=0.542.

Discussion

In this cross-sectional study of women with SLE and RA we explored the relationship between these two inflammatory rheumatic diseases and body composition. As fat mass, lean mass and fat distribution differ among different ethnic groups (24), only Caucasians were included.

The main finding is that Caucasian women with inflammatory rheumatic diseases are more likely to have abnormal body composition phenotype than non-inflammatory controls, with some differences existing between SLE and RA. The amount of body fat is higher in RA women than in controls, regardless of having comparable mean BMI and a similar proportion of overweight and obesity according to the BMI cutoffs. BMI does not discriminate between lean and fat mass, and the cutoff points of BMI for RA patients have been recently challenged (25). In RA, not only the amount of FM, but also the distribution of adipose tissue is altered. Indeed, the prevalence of central obesity is significantly higher in women with RA as compared to the control group. These findings may have important clinical implications as adipose tissue is a major source of adipokines involved in several metabolic and inflammatory processes. Moreover, abdominal obesity is a surrogate marker of visceral fat accumulation (26) and a well documented predictor of cardiovascular events (27). Abdominal obesity is linked to the insulin resistance observed frequently in RA patients (14, 28) and, while the effective control of inflammatory activity improves insulin resistance (29), serum levels of adipokines seem to remain largely independent of short-term RA disease activity control (30, 31).

The mean fat-free mass was similar in our patients with inflammatory diseases and non-inflammatory controls. However, a significantly greater proportion of SLE (17.4%) and RA (10.1%) women could be classified as sarcopenic, as compared with non-inflammatory controls (6.5%). The excessive waste of fat-free mass found in SLE and RA has been attributed to disease activity and decreased physical activity. Additionally, the catabolic effect of high corticosteroid doses (32), such as those used in severe lupus, might have contributed to the more frequently sarcopenia observed in SLE patients. Reduced lean mass in combination with increased fat mass, a condition that is known as obese sarcopenia, was previously reported in older RA patients (10). Yet, we found obese sarcopenia as frequent in SLE (6.5%) as in RA (5.6%) women and absent among controls. In the general population, obese-sarcopenia is found in older people and is associated with worse physical function (33). Controversy exist wheater cardiovascular risk is increased in rheumatic patients with sarcopenia (34).

There is a close relationship between body fat and demographic and lifestyle characteristics. Nevertheless, disease activity and pharmacotherapy also affect the percentage of body fat. The percentage of FM is lower in patients with higher disease activity, which may be explained by the catabolic effect of pro-inflammatory cytokines, in particular IL-6 and TNF, inducing weight and appetite loss (35, 36). In fact, despite not having measured cytokine levels, it has been shown that TNF levels are increased in SLE and in RA as compared with controls (37, 38) and there is evidence that an hypermetabolic state is associated with high levels of TNF, IL-1, and very active disease (39, 40). However, similarly to other authors (41), we could not depict an independent effect of TNF inhibition on total fat mass percentage in moderately active RA.

The relationship of CRP and FM percentage is inverse in lupus patients and independent of SLEDAI. As CRP is not usually increased in active SLE, and no significant correlation could be detected between CRP and SLEDAI (data not shown), this finding may possible reflect higher CRP levels related to the adipose tissue itself. Current corticosteroid dose was not identified as an independent predictor of FM percentage. Corticosteroids are used in more active disease and, by controlling inflammation, low doses may counterbalance the catabolic effect of pro-inflammatory cytokines.

Surprisingly, the observations on the amount of fat mass not associated with disease duration, disease severity, exercise, DMARD or immunosuppressant use, a finding that deserves further research. A possible explanation relates to the limitations of the cross sectional study design that cannot accurately assess the cumulative effect of disease, medication and exercise over time. Another limitation of the present study is the lack of dietary intake evaluation, a known risk factor for altered body composition in the general population (42). We cannot rule out differences in nutrient intake related to medication or disease activity as decreased appetite and reduced protein intake may occur in highly active disease (41), but is usually normal when disease activity is controlled (39). Additionally, 59.2% of patients were on steroids and even the small mean prednisolone dose could increase appetite. Nevertheless, our study population comprised a wide spectrum of disease activity which might minimise the effect of inflammatory activity on appetite, thus making significant differences in food or nutrient intake between women with inflammatory disease and controls from the same social and cultural background less likely. Another relevant variable in this context is physical activity. Physical activity did not emerge as having a relevant impact on FM. However, 81.5% of patients were sedentary, which means that the physically active group may have been too small to allow detection of a significant effect.

The rationale for a comparative study of two inflammatory rheumatic diseases was based on the assumption that different inflammatory settings could have a diverse effect on the study parameters. Both diseases are associated with altered body composition phenotype, yet some differences could be detected between SLE and RA. While in RA an increase in FM and central obesity prevails, more SLE women presented decreased lean mass. Nevertheless, the independent negative effect of disease activity on body fat mass was similarly observed in both diseases. These findings provide further support to the hypothesis that the alterations in body composition observed in inflammatory rheumatic diseases are partly explained by the inflammatory process itself.

In conclusion, women with SLE and RA exhibit changes in body composition more frequently than non-inflammatory controls, which may, at least in part, be a consequence of the inflammatory burden of the disease and its treatment. BIA and waist circumference are inexpensive and easy to perform assessments that provide important clinical information in SLE and RA patients. Conversely, weight and BMI do not discriminate between fat and fat-free body mass, which makes them less accurate measures for patients with chronic inflammatory rheumatic diseases.

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PARTE III

Lymphotoxin-α 252 A>G Polymorphism: A Link Between Disease Susceptibility and Dyslipidemia in Rheumatoid Arthritis?

Lymphotoxin-α 252 A>G Polymorphism: A Link Between Disease Susceptibility and Dyslipidemia in Rheumatoid Arthritis?

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ABSTRACT. ***Objective.*** Rheumatoid arthritis (RA) is associated with higher levels of inflammatory mediators and with a more atherogenic lipid profile. Dyslipidemia can be present years before arthritis develops. Lymphotoxin-α (LTA) is a cytokine that mediates proinflammatory responses while also participating in lipid homeostasis, and its transcriptional activity is in part genetically determined. We examined the role of the single-nucleotide polymorphism at position 252 of the LTA gene in the genetic background of RA and dyslipidemia.

Methods. The association between the LTA 252 A>G polymorphism and disease status was examined in a nested case-control study of 388 patients with RA and 269 unrelated healthy controls, all white. Demographics and disease features were assessed, fasting lipids measured, and the use of lipid-lowering agents evaluated.

Results. The LTA 252 A allele was more frequent in cases compared to controls (70.5% and 64.3%, respectively; p = 0.018, OR 1.325, 95% CI 1.049–1.675), as well as the A/A genotype (50.8% vs 43.5%; p = 0.025). The A/A genotype was independently associated with dyslipidemia in patients, but not in controls. Patients with RA who had the LTA 252 G/G genotype were younger at disease onset and had higher C-reactive protein (CRP) levels.

Conclusion. We found the LTA 252 A allele to be associated with an increased risk for developing RA in whites. The LTA 252 A/A genotype translates to a phenotype more prone to dyslipidemia, and the G/G genotype to a phenotype with earlier onset of disease and higher levels of CRP, when RA does occur. These observations highlight a possible common genetic predisposition to RA and dyslipidemia. (First Release April 1 2011; J Rheumatol 2011;38:1244–9; doi:10.3899/jrheum.101170)

Key Indexing Terms:
RHEUMATOID ARTHRITIS GENETICS INFLAMMATION LYMPHOTOXIN-α
DYSLIPIDEMIA SINGLE-NUCLEOTIDE POLYMORPHISM

Lymphotoxin-α (LTA) is a member of the tumor necrosis factor (TNF) superfamily, which is primarily synthesized by T and B lymphocytes. This cytokine shares structural and functional similarities with TNF and acts through its receptors to mediate proinflammatory and immunological responses[1]. Apart from its importance in inflammation, LTA forms heterotrimers with lymphotoxin-ß (LTB), which then interact with a specific receptor, the lymphotoxin receptor-ß. This signaling pathway seems to participate in the regulation of various processes, including lymphoid organ development, lipid homeostasis, and atherosclerotic plaque growth[2,3,4,5].

Rheumatoid arthritis (RA) is a well known inflammatory rheumatic disease characterized by synovial hyperplasia and by an excess of inflammatory cells, which in turn cause the degradation of cartilage and bone, resulting in the destruction of joints and progressive functional impairment. Cytokines have been considered pivotal in mediating pathophysiologic events in RA, a view that is supported by their increased levels in both the serum and the synovial fluid of patients with active disease[6,7] and by the clinical benefit of cytokine inhibition[8].

There is growing evidence that patients with RA display a more atherogenic lipid profile. Not only has the association between dyslipidemia and acute-phase response been demonstrated, but it has also been observed that dyslipi-

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demia is present years before arthritis develops, which cannot be explained by inflammation itself[9,10].

The variation in the production capacity of cytokines is in part genetically determined. TNF and LTA genes are located within the MHC III region of chromosome 6, in close linkage to the class II genes. LTA gene polymorphisms have been associated with variations in the transcriptional activity of LTA[11] and in circulating concentrations of C-reactive protein (CRP)[12] and TNF[13]. Additionally, a relationship between the GG genotype in the LTA single-nucleotide polymorphism (SNP) at position 252 and hypertriglyceridemia and decreased high-density lipoprotein (HDL) cholesterol was reported in Korean men[14]. Moreover, a recent larger study showed the LTA 252 variant allele to be more frequent in patients with RA than in healthy controls and to be associated with a tripled risk of myocardial infarction[15].

If genetic variants of LTA are important determinants of inflammation and also of dyslipidemia, this could have important implications for understanding the link between these 2 conditions in RA. For this reason we examined the role of the potentially functional LTA 252 A>G polymorphism in RA and whether this polymorphism is related to the severity of the disease and to dyslipidemia, in a nested case-control study. We also attempted to correlate this SNP with serum levels of proinflammatory mediators and fasting lipids.

MATERIALS AND METHODS

Study sample. Consecutive white patients attending the rheumatology outpatient clinics of Hospital Santa Maria, Lisbon, and Hospital Garcia de Orta, Almada, Portugal, on a regular basis and satisfying the 1987 American College of Rheumatology criteria for RA[16] were enrolled in this nested case-control study from a large cohort of patients with RA. Exclusion criteria were ancestry other than white, pregnancy, and breastfeeding. The control group consisted of unrelated healthy white volunteers. Eligibility criteria for controls were the same as for cases, except that controls must not have been diagnosed with RA or other inflammatory disease.

All patients underwent a clinical and laboratory evaluation and the following information was collected: age, sex, body mass index (BMI), age at RA diagnosis, positivity for rheumatoid factor, extraarticular manifestations (rheumatoid nodules, interstitial lung disease, pericardial or pleural effusion, Sjögren's syndrome, vasculitis, and amyloidosis), previous orthopedic surgeries due to RA (including total joint replacement or arthrodesis), current and past medications, comorbidities, and smoking habits. RA disease characteristics and comorbidities were validated by medical records review. Erythrocyte sedimentation rate (ESR) was measured, 28 joints were examined for tenderness and swelling, and the Disease Activity Score (DAS28) ESR[17] was calculated. Functional status was evaluated using the Stanford Health Assessment Questionnaire Disability Index (HAQ)[18]. Recent (< 6 months) plain radiographs of hands and feet were reviewed for the presence of erosions. A venous blood sample was collected into EDTA-containing tubes and preserved at –80°C until DNA extraction. An additional blood sample was obtained from 204 participants for measurement of fasting lipid profile, CRP, LTA, TNF, soluble TNF receptor I (sTNFR I), and interleukin 6 (IL-6). Since men and women have different frequency distribution of plasma lipids, this confirmatory group comprised women only; the sample size has a 95% power to detect a difference > 10% in lipid level at a significance level of 0.05.

Three outcomes were defined as surrogate markers of RA severity: (1) the presence of erosions; (2) extraarticular manifestations; and (3) HAQ score > 1. Dyslipidemia was defined as total cholesterol ≥ 200 mg/dl or low-density lipoprotein (LDL) cholesterol ≥ 130 mg/dl or high-density lipoprotein (HDL) cholesterol < 50 mg/dl for women or < 40 mg/dl for men or triglycerides ≥ 150 mg/dl, or the use of lipid-lowering agents.

The protocol was approved by the local Ethics Committee and written informed consent was obtained from all participants.

DNA extraction. DNA was extracted from whole blood using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), according to manufacturer's instructions.

Genotyping. The LTA 252 A/G polymorphisms were determined by restriction fragment length polymorphism (RFLP) analysis, using the forward primer 5'- CCG TGC TTC GTG CTT TGG ACT A -3' and the reverse primer 5'- AGA GCT GGT GGG GAC ATG TCT G -3' (Invitrogen, San Diego, CA, USA).

The polymerase chain reaction was performed in a 20-μl reaction mixture containing 100 ng genomic DNA, 40 μM dNTP (Invitrogen), 2 mM $MgCl_2$ (Finnzymes, Espoo, Finland), 0.4 μM of each primer, and 0.02 U/μl of the enzyme Phusion™ DNA Polymerase (Finnzymes). The cycle conditions were denaturation of the template DNA in a cycle of 98°C during 30 s; amplification of target DNA for 35 cycles of 98°C for 10 s, 71°C for 10 s, and 72°C for 12 s; and final extension in a cycle of 72°C for 7 min.

The amplified products [741 base pairs (bp)] were digested at 37°C with NcoI enzyme (New England Biolabs, Hitchin, UK) and evaluated in 2% agarose gels. Fragments with 545 and 196 bp were defined as G/G genotype; 741, 545, and 196 bp as A/G genotype; and undigested fragments with 741 bp as A/A genotype. Genotyping was repeated in 10% of the samples for purposes of quality control.

Cytokine measurement. The cytokines LTA, TNF, sTNFR I, and IL-6 were analyzed using the Bender MedSystems (Vienna, Austria) bead-based assay for quantitative detection of soluble human analytes by flow cytometry. The protocol was performed according to the manufacturer's instructions.

Statistical analysis. Continuous variables are presented as means ± SD or medians and 25th to 75th percentiles, depending on whether the data were normally distributed. Categorical variables are reported as absolute values and proportions.

Consistency of genotype frequencies with the Hardy-Weinberg equilibrium was examined using a chi-squared goodness-of-fit test on a contingency table of observed compared with expected genotype frequencies. Power was calculated under the log-additive model at 2-sided $\alpha = 0.05$, using Quanto version 1.2.4[19].

Association of LTA 252 A>G alleles and genotypes with the diagnosis of RA was analyzed by chi-squared tests. Within RA we assessed the independent relationship between genotypes and disease severity and dyslipidemia using logistic regression. If a significant association was identified, logistic regression analysis was also used to adjust for confounders. Student's t test or the Mann-Whitney U test was used to compare levels of lipids and inflammatory mediators between cases and controls and 1-way ANOVA and Kruskal-Wallis tests were used to assess differences among genotypes, as appropriate.

Statistical analysis was carried out using SPSS version 17.0 for Windows software (SPSS Inc., Chicago, IL, USA), and a 2-tailed p value < 0.05 was considered significant.

RESULTS

Association of LTA 252 polymorphism with RA diagnosis. The study group consisted of 657 white participants, 388 patients with RA and 269 unrelated healthy controls. Women were 88.7% of cases and 90.3% of controls. The genotype frequency of the LTA 252 A>G polymorphism was in agreement with that predicted by the Hardy-Weinberg

equation in both the RA (p = 0.30) and the control population (p = 0.12). The distribution of genotypes and alleles in patients and controls is shown in Table 1.

In RA we identified 50.8% A/A homozygotes, 39.4% A/G heterozygotes, and 9.8% G/G homozygotes, resulting in allele frequencies of 70.5% and 29.5% for A and G alleles, respectively. The common allele frequency was significantly higher in RA (p = 0.018) and the A/A genotype was associated with an increased likelihood of having RA (OR 1.772, 95% CI 1.076–2.921, p = 0.02). After correction for age and sex, this association remained statistically significant (OR 2.293, 95% CI 1.226–4.089, p = 0.009). The sample size had an 80% power to detect a size-effect ≥ 1.4 under a log-additive genetic model at a 0.05 significance level for a risk allele frequency of 64%.

LTA 252 genotypes and RA characteristics. Characteristics of RA subjects are presented in Table 2. Patients carrying the LTA G/G genotype were younger (p = 0.001) and also younger at onset of RA (p < 0.0001) than those carrying A/A or A/G genotypes, regardless of similar disease duration. Fewer patients with the A/A genotype were smokers (p = 0.02), otherwise the 3 groups presented similar median disease activity, proportion of rheumatoid factor positivity, and joint surgeries, as well as therapeutic options (use of corticosteroids, methotrexate, or biological agents). The prevalence of comorbid conditions did not differ across LTA genotypes.

The severity of RA was analyzed for the presence of erosions, extraarticular features, and increased disability (HAQ score > 1). Hands and feet radiographs were available for review in 72% of patients. In univariate logistic regression the presence of erosions was more likely in older patients (p < 0.0001), with older age at RA onset (p = 0.03), longer disease duration (p = 0.0001), being a woman (p = 0.03), and

Table 1. Lymphotoxin-α (LTA) 252 genotype and allele frequencies. Values calculated with the G/G genotype as reference.

	Patients with RA, n = 388	Control Subjects, n = 269	p	OR (95% CI)
LTA 252 genotypes				
A/A	197 (50.8%)	117 (43.5%)	0.025	1.772 (1.076–2.921)
A/G	153 (39.4%)	112 (41.6%)	0.160	1.438 (0.867–2.386)
G/G	38 (9.8%)	40 (14.9%)		
Allele frequency				
A allele	547 (70.5%)	346 (64.3%)	0.018	1.325 (1.049–1.675)
G allele	229 (29.5%)	192 (35.7%)	0.018	0.754 (0.597–0.954)

Table 2. Demographic and clinical characteristics of patients with rheumatoid arthritis (RA) according to the lymphotoxin-α A>G genotype. Data are expressed as median (25th–75th percentile) or proportions (%).

Characteristics	RA Total, n = 388	A/A, n = 197	A/G, n = 153	G/G, n = 38	p
Women	344 (88.7)	173 (87.8)	137 (89.5)	34 (89.5)	0.87
Age, yrs	58 (48–67)	59 (49.5–67)	59 (50.5–67)	45 (35–59)	0.001
Body mass index, kg/m^2	26.7 (23.1–29.7)	27.2 (23.5–29.2)	26.6 (23.5–30.6)	24.7 (21.4–28.1)	0.19
Waist, cm	87.2 (11.3)	87.5 (10)	87.6 (12)	85.9 (15)	0.57
Current smoker	52 (13.4)	12 (6.1)	34 (22.2)	6 (15.8)	0.02
Age at RA onset	44 (32–55)	45 (34.5–55.5)	46 (33–56)	34 (22–43)	< 0.0001
Disease duration, yrs	10 (4.4–18)	10 (4.7–19.3)	10 (4.2–18.9)	10 (4.1–18.2)	0.88
Rheumatoid factor-positive	284 (73.2)	139 (70.6)	116 (75.8)	29 (76.3)	0.58
DAS28	4.1 (3.0–5.3)	3.9 (2.9–5.2)	3.9 (3.0–5.5)	4.5 (3.5–5.1)	0.65
HAQ	1.125 (0.5–1.87)	1 (0.5–1.75)	1.25 (0.62–1.87)	1.25 (0.25–1.84)	0.58
Erosions, n = 284	235 (82.7)	133 (87.5)	85 (80.2)	17 (65.4)	0.015
Extraarticular features	96 (24.7)	52 (26.4)	36 (23.5)	8 (21.1)	0.71
Joint surgery	52 (13.4)	22 (11.2)	28 (18.3)	2 (5.3)	0.34
Corticosteroids	262 (67.5)	136 (69)	105 (68.6)	21 (55.3)	0.46
Methotrexate	292 (75.3)	146 (74.1)	121 (79)	25 (65.8)	0.43
Biologics	74 (19)	37 (18.8)	32 (20.9)	5 (12.8)	0.51

DAS: Disease Activity Score; HAQ: Health Assessment Questionnaire.

having the A/A genotype (OR 3.706, 95% CI 1.447–9.448, p = 0.006). Factors identified to be significantly associated with both A/A genotype and erosions, thus acting as potential confounders, included age (p = 0.001 and p < 0.0001, respectively) and age at disease onset (p < 0.0001, p = 0.03). Following adjustment for confounders, the effect of genotype on erosions was no longer statistically significant (OR 1.799, 95% CI 0.600–5.397, p = 0.29). We did not find any statistically significant association between the A/A genotype and the presence of extraarticular manifestations (OR 1.345, 95% CI 0.580–3.121, p = 0.49) or disability (OR 1.031, 95% CI 0.510–2.084, p = 0.932) either in the crude model or following adjustment for potential confounders (extraarticular manifestations, OR 1.292, 95% CI 0.537–3.110, p = 0.56; disability, OR 1.290, 95% CI 0.620–2.682, p = 0.49).

LTA genotypes and dyslipidemia. Fifty patients (25.4%) with LTA 252 A/A, 27 (17.6%) with A/G, and 4 (7.7%) with G/G genotype had been previously diagnosed with dyslipidemia (p = 0.23) and were receiving treatment with lipid-lowering agents. As dyslipidemia could be underdiagnosed and/or undertreated, we measured fasting cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides in 204 participants (94 patients with RA and 110 controls, all women). Disease characteristics of this confirmatory group were similar to the whole population. Dyslipidemia was highly prevalent in both patients (75.5%) and controls (67.3%). In our confirmatory group, the presence of dyslipidemia was more likely in older participants (OR 1.047, 95% CI 1.022–1.072, p < 0.001), with higher BMI (OR 1.010, 95% CI 1.001–1.101, p = 0.04), and in those carrying the A/A genotype (OR 2.773, 95% CI 1.209–6.361, p = 0.01). Following adjustment for confounders (age and BMI), and in patients also for DAS28 and corticosteroid dose because active RA and steroids may alter lipid profile, the association between dyslipidemia and genotype remained significant only in patients with RA (Table 3). The A/A genotype was independently associated with dyslipidemia (OR 18.3, 95% CI 2.91–94.80, p = 0.002), as well as with higher levels of triglycerides (p = 0.01) in patients not treated with lipid-lowering agents. There was also a trend toward higher total cholesterol levels among patients carrying the A/A genotype (p = 0.08). HDL and LDL cholesterol were not significantly different among genotypes (Table 4).

Inflammatory markers. Plasma LTA levels were below the limit of detection in the majority of samples and could not be analyzed further. Plasma levels of CRP, TNF, sTNFR I, and IL-6 were compared in patients with RA and controls (Table 5). As expected, we found significantly higher CRP (10 ± 2 mg/l vs 3 ± 2.8; p < 0.0001), TNF (13.3 ± 30.3 pg/ml vs 5.1 ± 4.9 pg/ml; p < 0.0001), sTNFR I (1.8 ± 1.2 pg/ml vs 1.5 ± 0.9; p = 0.07), and IL-6 (4.8 ± 2.4 pg/ml vs 2.4 pg/ml; p = 0.04) levels in patients compared to healthy controls.

Table 3. Association of dyslipidemia with lymphotoxin-α 252 genotypes in patients with rheumatoid arthritis. OR adjusted for age, body mass index, current prednisone dose, and 28-joint count Disease Activity Index (DAS28).

	Crude OR (95% CI)	p	Adjusted OR (95% CI)	p
Genotype				
A/A	18.00 (4.01–80.71)	< 0.001	18.30 (2.91–94.80)	0.002
A/G	5.85 (1.46–23.37)	0.012	5.10 (0.89–29.15)	0.08
G/G (reference)	1		1	
Age	1.039 (1.004–1.075)	0.030	—	
Body mass index	1.148 (1.023–1.289)	0.019	—	
Prednisone dose	1.055 (0.926–1.218)	0.095	—	
DAS28	0.722 (0.507–1.029)	0.072	—	

Patients with the G/G genotype presented CRP levels almost 3 times higher (13.1 ± 8.9 mg/l) than those with the A/A genotype (5.3 ± 4.9 mg/l; p = 0.007) and this difference remained significant after adjustment for disease activity (p = 0.001). No significant differences in TNF or IL-6 levels were identified among genotypes in patients or in controls.

DISCUSSION

RA is both genetically and clinically a heterogeneous disease. Although its causes remain largely unknown, the importance of proinflammatory cytokines in the pathophysiology of RA has been emphasized recently. Polymorphisms in cytokine genes are associated with different cytokine transcriptional levels, and these variations might affect not only the frequency of the disease, but also its phenotypic expression. All these clues point toward functional cytokine gene polymorphisms being potential additional risk factors for RA.

We report a significant association of the LTA A allele with RA in whites. In particular, the A/A genotype was associated with almost doubled odds of diagnosis of RA. However, the disease risk allele was not associated with surrogate markers of RA severity.

Our study population comprised a relatively large and homogeneous group of whites mostly from south Portugal. Patients with RA were followed regularly in a cohort study at the participating institutions for several years, the disease was well characterized, and detailed clinical information was confirmed from patient files. The prevalence of the variant G allele in healthy controls was identical to that reported in large European control populations[12], but lower when compared to Asian populations[20,21]. Nevertheless, this study has several limitations, including the fact that we did not test for other potentially relevant polymorphisms of the TNF cluster that might be in linkage disequilibrium with the studied one.

There are few studies addressing the LTA 252 A>G SNP

Table 4. Lymphotoxin-α 252 A>G genotypes and fasting lipid levels in participants not receiving lipid-lowering therapy. Results are means ± SD.

Lipid Levels (mg/dl)	Patients with RA			Healthy Control Population		
	A/A, n = 34	A/G, n = 27	G/G, n = 13	A/A, n = 25	A/G, n = 38	G/G, n = 21
Total cholesterol	212 ± 32	200 ± 37	197 ± 38	200 ± 32	205 ± 32	197 ± 26
HDL	65 ± 24	64 ± 14	64 ± 13	59 ± 13	62 ± 11	65 ± 15
LDL	129 ± 24	120 ± 33	113 ± 32	124 ± 33	127 ± 26	119 ± 22
Triglycerides	108 ± 41*	98 ± 36†	81 ± 24	94 ± 31	94 ± 39	92 ± 47

* Adjusted $p_{A/A \text{ vs } G/G} = 0.01$ and † $p_{A/G \text{ vs } G/G} = 0.05$. HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Table 5. Serum levels of inflammatory mediators in patients and healthy controls according to genotype.

Serum Levels	Patients with RA			Controls		
	A/A, n = 45	A/G, n = 36	G/G, n = 13	A/A, n = 33	A/G, n = 51	G/G, n = 26
ESR, mm/h	35 ± 18	39 ± 30	41 ± 19	19 ± 10	21 ± 12	22 ± 16
CRP, mg/l	5.3 ± 4.9	15.1 ± 39	13.1 ± 8.9*	3.3 ± 2.8	2.7 ± 2.3	3.6 ± 3.6
TNF, pg/ml	10.5 ± 17.4	18.6 ± 44	7.9 ± 12.5	6.2 ± 5.9	4.8 ± 5	4.3 ± 3.6
sTNFR I, pg/ml	1.73 ± 0.9	1.90 ± 1.4	1.79 ± 0.8	1.51 ± 1.0	1.33 ± 0.7	1.91 ± 0.8†
IL-6, pg/ml	3.6 ± 9.1	7.6 ± 17.5	LOD	LOD	LOD	LOD

* Adjusted $p_{G/G \text{ vs } A/A} = 0.001$; † $p_{G/G \text{ vs } A/G} = 0.02$. ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; TNF: tumor necrosis factor; sTNFR I: soluble TNF receptor I; LOD: concentrations below the limit of detection.

in RA, most of them with a small number of patients, which might have contributed to the conflicting results. Takeuchi, *et al* reported an increased frequency of the LTA A allele in Japanese DRB1*0405-positive patients[20], apparently independent of MHC class II genes, in a study that included 103 patients with RA. In contrast, Panoulas, *et al* recently found increased carriage of the G allele among 388 British patients[15]. Others report no relation of this SNP with RA[22,23]. In addition to sample size issues, a possible explanation for these different results is that genetic susceptibility related to the LTA gene may vary in different ethnic groups. In addition, different inclusion criteria and lack of homogeneity of the studied populations might contribute to further confounding factors. Another hypothesis is that differences are due to linkage disequilibrium with other genes of the MHC more relevant to RA susceptibility.

Although LTA 252 A>G is an intronic polymorphism and some controversy exists whether this is a functional polymorphic site, there is compelling evidence suggesting that the LTA 252 G/G genotype is linked to enhanced expression of LTA and higher serum concentrations of inflammatory markers[11,12,13,24]. This is important, as the increased proinflammatory environment could influence the phenotypic expression of RA. We observed a strong association between the LTA 252 G/G genotype and younger age at RA onset that has not been previously reported. Interestingly, the influence of the allelic variation of the LTA 252 on the mean age at onset of psoriasis was described by Balding, *et al* in patients with psoriatic arthritis[25], thus suggesting the influence of the G/G genotype on the beginning of the disease at youngest age. We also measured inflammatory mediators in a confirmatory group and found higher CRP levels in patients possessing the G/G genotype, but no significant differences could be detected in TNF, sTNFR I, or IL-6 levels. CRP levels are influenced by genetic factors and the LTA 252 G allele was associated with high levels in population studies[11,26]. The relatively small number of individuals carrying the G/G genotype as well as the high dispersion of measured values could have contributed to the lack of association with other inflammatory mediators. Another limitation is the cross-sectional design of the study and the fact that patients were at different stages of the disease and under treatment for years.

We found a strong association between dyslipidemia and the A/A genotype in patients with RA that could not be explained by other factors, but this was not observed in the control population. In particular, triglyceride levels were higher among patients carrying the A allele, independent of age, disease activity, or corticosteroid use. Panoulas, *et al*[15] also found significantly higher total cholesterol, as well as a trend toward higher LDL and triglyceride levels in patients with RA carrying the LTA 252 A/A genotype. Moreover, higher fasting triglycerides were previously reported in healthy white men possessing the LTA A allele[27] in contrast

with results from Korean men[14], but sex and ethnic-related variations could account for the different results. Together, these observations suggest that the dyslipidemia that accompanies and even precedes RA is not only a consequence of inflammation but is also genetically determined. However, these results need replication in larger population samples.

Our study shows that the LTA 252 A allele is associated with increased risk of developing RA in Portuguese whites. Moreover, the A/A genotype translates to a phenotype that is more prone to dyslipidemia, raising the possibility that a genetic element contributes to the disordered lipid metabolism encountered in patients with RA as well as in individuals who eventually develop RA. This SNP also influences age of disease onset and levels of CRP, but it does not seem to be a major determinant of RA severity. Although our study involved a relatively large number of subjects, replication is needed in other cohorts for testing the robustness of these results. If confirmed, these findings will contribute to understanding the underlying mechanisms for the cardiovascular burden in RA.

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PARTE IV

Hemorheological parameters are related to subclinical atherosclerosis in systemic lupus erythematosus and rheumatoid arthritis patients.

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Hemorheological parameters are related to subclinical atherosclerosis in systemic lupus erythematosus and rheumatoid arthritis patients

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ABSTRACT

Objectives: Rheological characteristics of blood are strongly linked to atherothrombosis in the general population, but its contribution to atherosclerosis in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) is currently unclear. This work examines the relationship between blood rheology, traditional cardiovascular (CV) risk factors, inflammation and subclinical atherosclerosis in SLE and RA.
Methods: Whole blood viscosity (WBV), plasma viscosity (PV), erythrocyte deformability (ED), aggregation (EA) and erythrocyte NO production were measured in 197 patients (96 SLE and 101 RA) and compared to 97 controls, all females without previous CV events. Clinical information was obtained and fasting lipids and acute phase reactants were measured. The relationship between hemorheological parameters, CV risk factors and inflammation was assessed in patients and the impact of these variables on carotid intima-media thickness (cIMT) was evaluated in univariate followed by multivariate regression analyses.
Results: WBV and ED are significantly lower in patients, while EA is elevated as compared with controls. Hemorheological disturbances correlate with CV risk factors and markers of inflammation and are more profound in patients with metabolic syndrome. Multivariable analysis showed that menopause (OR 34.72, 95%CI 4.44–271.77), obesity (OR 4.09, 95%CI 1.00–16.68) and WBV (OR 3.98; 95%CI 1.23–12.83) are positively associated whereas current corticosteroid dose (OR 0.87; 95%CI 0.78–0.98), and erythrocyte NO production (OR 0.16; 95%CI 0.05–0.52) are negatively associated with cIMT.
Conclusion: Disturbed hemorheological parameters in SLE and RA women are related to the presence of CV risk factors and inflammation. WBV and erythrocyte NO are independently associated with the early stages of atherosclerosis.

1. Introduction

Over the last decades there have been several studies supporting the notion that patients with systemic lupus erythematosus (SLE) and patients with rheumatoid arthritis (RA) are at increased risk for ischemic heart disease and stroke [1,2]. Apart from traditional cardiovascular (CV) risk factors, a multitude of disease related features are undoubtedly involved in the CV risk of these patients [3,4].

Hemostatic and hemorheological factors are clearly linked to vascular ischemic events in the general population [5–7] and in the Edinburgh Artery Study blood viscosity and fibrinogen were found to be related to CV events at least as strongly as traditional risk factors [8]. In fact, by favoring a prothrombotic milieu, promoting endothelial damage and diffuse intimal thickening [9–11], rheological characteristics of blood may contribute to atherogenesis. In SLE and RA there is preliminary evidence suggesting disturbed blood rheology [12–14] which can be hypothesized to contribute to atherosclerosis in these patients.

The aim of the present study was to characterize the hemorheological profile of women with SLE and with RA and to investigate the relationship between altered hemorheological parameters, inflammation, traditional CV risk factors, and subclinical atherosclerosis.

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2. Patients and methods

2.1. Study design and patient population

We studied 96 adult women with SLE, 101 with RA and 97 healthy controls without overt atherosclerotic disease. Eligible patients met the ACR 1997 classification criteria for SLE or the 1987 revised criteria for RA and were followed on a regular basis at the Hospital Garcia de Orta in Almada or Hospital Santa Maria in Lisbon. The control group consisted of women without inflammatory diseases recruited from the same clinics. Exclusion criteria included previous acute myocardial infarction, coronary artery revascularization procedures, transient ischemic attack, stroke, impaired renal function, pregnancy or breastfeeding. All participants gave written informed consent and the Ethics Committees of the participating institutions approved the study.

2.2. Data collection

All subjects were interviewed and examined by a rheumatologist using a standardized protocol. Information on demographic characteristics (age, race, education, menopause), traditional CV risk factors (smoking, height, weight, waist circumference and blood pressure), disease features (age at disease diagnosis, current disease activity and damage) and current medication (antihypertensive, lipid-lowering, aspirin, disease modifying anti-rheumatic drugs and corticosteroid dose) was obtained. Presence of antinuclear antibodies (ANA), IgM rheumatoid factor (RF,) lupus anticoagulant (LAC), anti-DNA, anti-SSA, anti-SSB, anti-RNP, anti-Sm, anti-cardiolipine (aCl) and anti-cyclic citrullinated peptide antibodies (CCP) was retrieved from patients' medical records. Fasting blood samples were collected for a laboratory examination that included glucose, lipids, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), hemoglobin, hematocrit, fibrinogen, whole blood viscosity (WBV), plasma viscosity (PV), erythrocyte aggregation (EA), erythrocyte deformability (ED) and erythrocyte nitric oxide (NO) production.

2.3. Definitions

Patients were considered hypertensive if blood pressure in repeated measurements was $\geq$140/90 mmHg or if they were taking antihypertensive medication. Dyslipidemia was defined as fasting cholesterol $\geq$200 mg/dl or LDL cholesterol $\geq$130 mg/dl or HDL cholesterol <50 mg/dl or triglycerides $\geq$150 mg/dl or use of lipid-lowering agents; diabetes as fasting glucose level $\geq$126 mg/dl or pharmacological treatment; obesity was defined as body mass index (BMI) $\geq$30 kg/m^2. Metabolic syndrome was diagnosed according to the joint definition of the International Diabetes Federation, National Heart Lung and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity and using waist circumference $\geq$80 cm as the threshold for abdominal obesity. Participants were classified as current smokers if they had smoked $\geq$1 cigarettes per day during the last month.

Disease activity was measured using the Systemic Lupus Disease Activity Index 2000 (SLEDAI-2K) [15] in lupus patients and using the Disease Activity Score 28 (DAS28) [16] in RA patients and categorized into remission (SLEDAI = 0 or DAS28 $\leq$ 2.6) or active disease (SLEDAI $\geq$ 1 or DAS28 > 2.6), in agreement to the SLEDAI and DAS28 definitions. SLE patients' damage was scored according to the Systemic Lupus International Collaborating Clinics/ACR Damage Index (SLICC) from 0 to 49. RA functional status was evaluated using the Stanford Health Assessment Questionnaire Disability Index (HAQ), a self-administered questionnaire which gives a score ranging from 0 to 3.

2.4. Hemorheological assessment

All measurements were performed within 2 h of sampling. WBV was evaluated in a Brookfield digital viscometer, using native blood samples anticoagulated with heparin lithium, submitted to low (22.5 s^{-1}) and high (225 s^{-1}) shear rates, both at a native (WBVn) and corrected hematocrit of 45% (WBVc), at 37 °C. PV was determined by Harkness method. EA was measured in an erythrocyte aggregometer at stasis (EA_0), during 10 s after dispersion of the blood samples, by rotation at shear stress of 600 s^{-1} (EA_1) and following adjustment of the hematocrit to 45% (EA_{1c}). ED was determined in a shear stress diffractometer (Rheodyn SSD laser diffractometer from Myrenne) at 0.6 Pa ($ED_{0.6}$), 6 Pa (ED_6) and 30 Pa (ED_{30}). Erythrocyte NO production was quantified using the amiNO-IV sensor (Innovative Instruments).

2.5. Subclinical atherosclerosis

To assess subclinical atherosclerosis patients underwent high-definition carotid ultrasonography performed by one of the authors (LMP) blinded to the diagnosis and using a Philips-ATL HDI 5000 equipment. Carotid intima-media thickness (cIMT) quantification was performed manually and according to the Mannheim Consensus. IMT was measured in the common carotid artery (CCA), 1 cm proximal to the carotid bifurcation and always in the distal arterial wall in a region free of plaque. Three measurements were performed in each side and the mean value of cIMT was obtained from 6 measurements (3 on left and 3 on right CCA). Patients were categorized according to cIMT in two groups: above and below the median cIMT. Carotid plaques were defined as localized thickness >1 mm and always with destruction of arterial layer structure.

2.6. Statistical analysis

Baseline characteristics are presented as means and standard deviation or percentages. Hemorheological parameters were compared across SLE, RA and controls using general linear model analysis adjusted for differences in baseline characteristics identified as possible confounders (e.g. associated also with the outcome of interest in univariate analysis at a $p \leq 0.10$). The same methodology was used to compare hemorheological parameters between patients in remission and with active disease and between those with and without the metabolic syndrome. Within the patient group, the relationship between altered hemorheological parameters and disease characteristics, markers of inflammation and CV risk factors was assessed using Spearman correlations. The associations between hemorheological parameters and subclinical atherosclerosis were explored through means of logistic regression models adjusted for age and race, with cIMT (below vs above the median) and presence of atherosclerotic plaques as dependent variables. To further assess the independence of the associations, other potential predictors were identified within the following domains (demographics, traditional CV risk factors, disease characteristics, medication) using univariate analyses. All variables found to have a bivariate relation (unadjusted $p \leq 0.10$) with the studied outcomes were considered possible predictors and entered into a domain-specific multivariate model. The selection of covariates was stepwise by backward selection within each domain-specific model. Covariates identified from each domain with a p value <0.05 were carried forward to the final multivariate model. Age and race were forced into the model. After the final selection, non significant variables removed were tested as confounders. Statistical analysis was performed using SPSS version 17.0 for Windows and p values <0.05 were considered statistically significant.

Table 1
Demographic data, CV risk factors, disease characteristics and medication of patient and control groups.

	SLE ($N=96$)	RA ($N=101$)	Controls ($N=97$)
Demographic data			
Mean age*, years	45.1 (13.3)	50.9 (13.3)	47.4 (13.4)
Caucasians*, %	88.5	89.1	97.9
Education, years	8.9 (4.8)	8.1 (5.1)	9.2 (5.2)
Menopause, %	47.8	60.4	53.1
Traditional CV risk factors			
Hypertension, %	49.0	49.5	37.1
Dyslipidemia, %	67.7	63.0	59.8
Diabetes, %	5.2	6.9	8.2
Current smoker, %	14.6	16.8	21.6
BMI, kg/m^2	26.9 (5.0)	28.0 (5.1)	25.8 (4.9)
Obesity, %	28.4	32.7	29.9
Metabolic syndrome, %	22.3	25.2	18.6
Characteristics of disease			
Duration, years	8.3 (6.6)	9.9 (7.6)	–
ANA+, %	100	–	
RF+ and/or anti-CCP+; %	–	90.1	
Activity index mean	SLEDAI 2K	DAS28	–
score	2.9 (4.5)	4.3 (1.3)	
Remission %	45.8	21.8	
Active %	54.2	78.2	
Damage/function	SLICC 0.73 ± 1.2	HAQ 1.16 ± 0.71	
Current medication			
Antihypertensives, %	37.5	30.7	21.6
Lipid lowering, %	25.0	16.8	18.6
Aspirin*, %	20.8	5.9	0.0
Corticosteroids, %	59.4	55.4	–
Antimalarials*, %	78.1	17.8	–
Methotrexate*, %	10.4	83.2	–

Results are expressed as crude means (standard deviation) or proportions (%).
BMI, body mass index; ANA, anti-nuclear antibodies; RF, IgM rheumatoid factor; CCP, cyclic citrullinated peptide; SLEDAI, systemic lupus erythematosus disease activity index; DAS28, disease activity score 28 joints; SLICC, systemic lupus international collaborating clinics/ACR damage index; HAQ, health assessment questionnaire.
* Statistically significant differences across the groups ($p<0.05$).

3. Results

3.1. Study population

One hundred and ninety seven patients (96 SLE and 101 RA women) and 97 controls were evaluated. Demographic characteristics, relevant comorbidities, current medication and disease activity are presented in Table 1. Women with SLE were younger ($p=0.01$) and both disease groups comprised fewer Caucasians than the control group ($p=0.03$). A total of 35 (36.5%) lupus patients were positive for antiphospholipid antibodies, 31 of whom tested positive for either IgM or IgG aCl and 20 had a positive LAC test. The use of aspirin and antimalarials was higher among women with SLE ($p<0.001$) and MTX use was more frequent in RA ($p<0.001$).

Table 3
Significant correlations between altered hemorheological parameters, demographic data, disease characteristics, CV risk factors and markers of inflammation in SLE and RA patients.

	WBVn 22.5 s^{-1}	EA_0	ED_6 Pa
Age	−0.03	0.07	0.16[a]
Disease duration	−0.06	0.08	0.18[a]
BMI	0.06	0.23[b]	−0.07
Waist circumference	0.07	0.28[c]	−0.17[a]
HDL cholesterol	−0.03	−0.18[a]	0.17[a]
Triglycerides	−0.01	0.22[b]	−0.07
ESR	−0.07	0.35[c]	0.08
CRP	−0.09	0.26[c]	−0.05
Hematocrit	0.61[c]	−0.17[a]	0.11
SLEDAI	−0.23[a]	0.17	−0.05

BMI, body mass index; HDL, high density lipoprotein; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; SLEDAI, systemic lupus erythematosus disease activity index.
[a] $p<0.05$.
[b] $p<0.01$.
[c] $p<0.001$.

3.2. Hemorheological parameters in patients and controls

Considering the different baseline characteristics between patients and control subjects, the results of the hemorheological parameters were adjusted for race, as this was identified as a possible confounder. Hematocrit and whole blood viscosity at low shear rate, either native (WBVn) or corrected to a standard hematocrit of 45% (WBVc) were significantly lower in patients than in the control group (Table 2). Patients also presented lower erythrocyte deformability at 6 Pa (ED_6) and higher erythrocyte aggregation at stasis (EA_0). No significant differences in plasma viscosity, fibrinogen levels or erythrocyte NO production were found between patients and controls ($p=0.465$, $p=0.092$ and $p=0.397$, respectively). Compared to RA, SLE women exhibited higher blood viscosity and higher serum fibrinogen levels ($p<0.01$).

3.3. Correlates of altered hemorheological parameters in SLE and RA

Among patients, WBVn correlated positively with hematocrit and negatively with SLEDAI 2K (Table 3). WBVn 22.5 s^{-1} was lower in SLE patients positive for anti-SSB (6.40 ± 0.17 vs 6.80 ± 0.06;

Table 2
Hemorheological parameters in SLE, RA and control women.

	SLE $N=96$	RA $N=101$	Controls $N=97$	p value (disease vs control)	p value (SLE vs RA)
Hct (%)	39.9 (0.4)	39.0 (0.4)	41.8 (0.4)	<0.001	0.11
Fibrinogen, mg/dl	326 (12)	275 (11)	275 (12)	0.09	<0.01
PV	1.27 (0.00)	1.30 (0.00)	1.25 (0.00)	0.46	0.46
WBVn 22.5 s^{-1}	6.74 (0.05)	6.63 (0.05)	6.87 (0.05)	<0.01	0.14
WBVc 22.5 s^{-1}	6.29 (0.07)	6.09 (0.07)	6.42 (0.07)	0.01	0.04
WBVn 225 s^{-1}	4.29 (0.02)	4.22 (0.02)	4.26 (0.02)	0.89	0.03
WBVc 225 s^{-1}	3.86 (0.06)	3.80 (0.06)	3.96 (0.06)	0.12	0.40
EA_0	11.64 (0.23)	11.65 (0.23)	11.02 (0.23)	0.03	0.97
EA_1	17.94 (0.37)	18.15 (0.36)	17.34 (0.37)	0.12	0.68
EA_{1c}	19.02 (0.39)	18.92 (0.38)	18.12 (0.39)	0.10	0.85
$ED_{0.6}$	5.38 (0.34)	5.22 (0.33)	5.71 (0.34)	0.32	0.73
ED_6	41.56 (0.41)	42.25 (0.41)	43.25 (0.41)	<0.01	0.23
ED_{30}	53.09 (0.63)	53.44 (0.61)	54.55 (0.63)	0.09	0.69
NO	1.75 (0.07)	1.67 (0.06)	1.64 (0.07)	0.39	0.47

Results are expressed as estimated marginal means (standard error) adjusted for race.
Hct, hematocrit; PV, plasma viscosity; WBVn, whole blood viscosity native; WBVc, whole blood viscosity corrected to the hematocrit 45%; EA_0, erythrocyte aggregation at stasis; EA_1, erythrocyte aggregation during 10 s after dispersion of the blood samples; EA_c, erythrocyte aggregation corrected to 45% hematocrit; $ED_{0.6}$, erythrocyte deformability at 0.6 Pa; ED_6, erythrocyte deformability at 6 Pa; ED_{30}, erythrocyte deformability at 30 Pa; NO, nitric oxide.

$p = 0.03$) and anti-Sm (6.28 ± 0.22 vs 6.79 ± 0.06; $p = 0.02$) antibodies. EA was significantly correlated with several CV risk factors (BMI, waist circumference, HDL cholesterol, triglycerides) and with inflammation markers (hematocrit, sedimentation rate, and C-reactive protein). Lupus patients positive for LAC had increased EA either at stasis (EA_0 12.68 ± 0.55 vs 11.29 ± 0.30; $p = 0.03$) or after dispersion (EA_1 19.58 ± 0.86 vs 17.39 ± 0.47; $p = 0.03$). EA_1 was also higher in RA patients positive for RF and/or anti-CCP antibodies (EA_1 19.17 ± 0.40 vs 16.64 ± 1.21; $p = 0.05$). ED correlated positively with age, disease duration and HDL cholesterol and negatively with waist circumference.

With respect to medication, patients receiving corticosteroids or methotrexate had a lower WBV either native or after correction to a hematocrit of 45%. Aspirin use was associated with reduced ED_6 in RA (37.73 ± 1.71 vs 42.52 ± 0.43; $p = 0.008$), but not in SLE. The use of antimalarials, anti-hypertensive or lipid lowering medication was not associated with hemorheological parameters.

3.4. Hemorheological alterations associated with active disease and with the metabolic syndrome

One hundred and thirty one patients (66.5%) presented some disease activity while 66 (33.5%) were in remission according to the SLEDAI or the DAS28. Hematocrit (38.9 ± 0.4 vs 40.5 ± 0.5, $p = 0.01$) and WBVn (WBVn 22.5 s^{-1} 6.62 ± 0.05 vs 6.82 ± 0.06, $p = 0.01$ and WBVn 225 s^{-1} 4.22 ± 0.02 vs 4.31 ± 0.03; $p = 0.01$) were lower in active disease as compared with remission. Hematocrit corrected WBVc22.5 s^{-1} was also lower in active disease (6.08 ± 0.06 vs 6.38 ± 0.09, $p < 0.01$), but no differences were observed regarding red cell aggregation, deformability, serum fibrinogen concentrations or erythrocyte NO in relation to disease activity.

Metabolic syndrome was diagnosed in 24% of the patients and associated with higher WBVn (WBVn 22.5 s^{-1} 6.83 ± 0.08 vs 6.65 ± 0.04; $p = 0.04$ and WBVn 225 s^{-1} 4.35 ± 0.03 vs 4.23 ± 0.18; $p < 0.01$), increased EA (EA_1 18.96 ± 0.59 vs 17.64 ± 0.31; $p = 0.04$ and EA_{1c} 20.11 ± 0.58 vs 18.44 ± 0.32; $p = 0.01$) and reduced ED (ED_6 40.36 ± 0.64 vs 42.46 ± 0.34; $p < 0.01$ and ED_{30} 51.28 ± 1.0 vs 54.27 ± 0.55; $p = 0.01$). As shown in Fig. 1, disease activity and metabolic syndrome had a potentiating effect on EA and ED and the presence of metabolic syndrome counteracted the effect of inflammation on WBV.

3.5. Subclinical atherosclerosis and hemorheological parameters

The proportion of women presenting carotid plaques was comparable in SLE, RA and controls (14.5%, 14% and 13%, respectively), as well as the mean cIMT (SLE 0.696 ± 0.19 mm, RA 0.769 ± 0.39 mm and controls 0.673 ± 0.02 mm; $p = 0.17$). In the patient group, atherosclerotic plaques were associated with the following hemorheological parameters: EA_1 (OR 1.16; 95%CI 1.01–1.32) and erythrocyte NO production (OR 0.54; 95%CI 0.29–0.99). Also identified as candidate predictors of plaque was age (OR 1.09; 95%CI 1.03–1.15), age at disease diagnosis (OR 1.08; 95%CI 1.03–1.13), active disease (OR 7.11; 95%CI 2.15–23.51), hypertension (OR 4.83; 95%CI 1.47–15.87), diabetes (OR 7.07; 95%CI 1.29–38.53), and current use of corticosteroids (OR 0.33; 95%CI 0.11–1.00). Given the limited number of patients with plaques, we were unable to perform multivariate analysis.

The hemorheological parameters associated with cIMT in univariate analysis were WBVn 22.5 s^{-1} (OR 2.30; 95%CI 1.16–4.56), ED_1 (OR 1.10; 95%CI 1.00–1.21) and NO (OR 0.55; 95%CI 0.30–1.00). Results or the multivariate analysis are shown in Table 4. In the final model adjusted for age (OR 1.04; 95% 0.96–1.14) and race (Caucasians as reference OR 0.53; 95%CI 0.07–3.94), the variables that best differentiated between patients with cIMT above and below the median were menopause (OR 34.72; 95%CI 4.44–271.77),

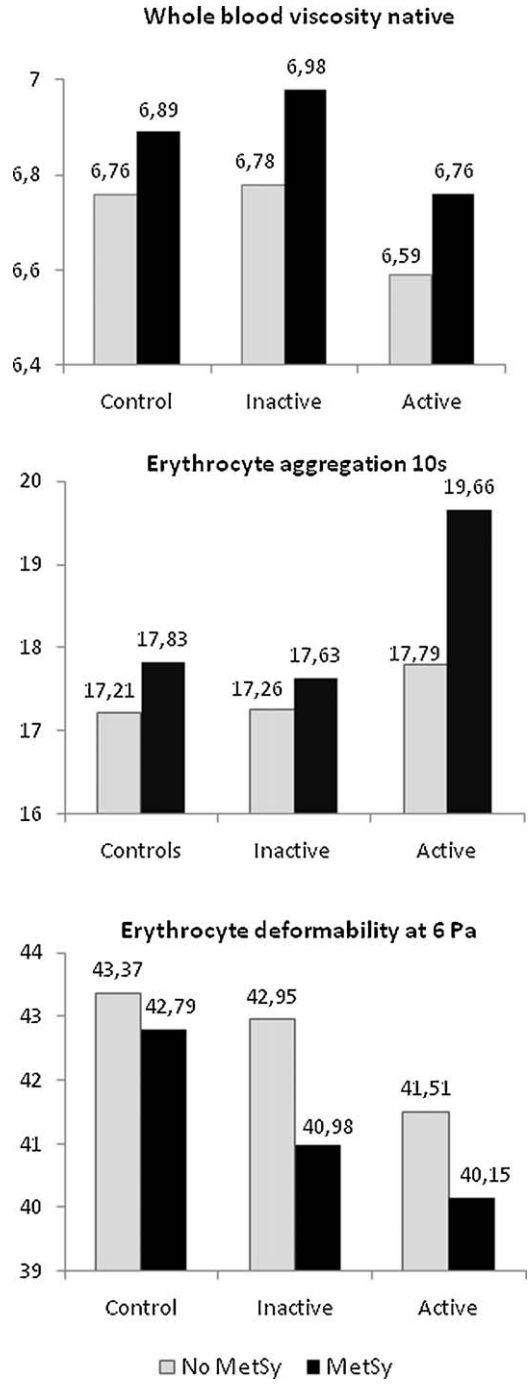

Fig. 1. Effect of disease activity and metabolic syndrome on blood viscosity, erythrocyte aggregation and deformability.

obesity (OR 4.09; 95%CI 1.00–16.68), current corticosteroid dose (OR 0.87; 95%CI 0.78–0.98;), WBVn 22.5 s^{-1} (OR 3.98; 95%CI 1.23–12.83) and NO (OR 0.16; 95%CI 0.05–0.52). The sensitivity and specificity of this model was 88.3% and 89.3%, respectively and the Nagelkerke pseudo R^2 was 0.741.

4. Discussion

Our work showed that whole blood viscosity and erythrocyte NO production are independently associated with subclinical atherosclerosis in SLE and RA women without previous CV events. Additionally, we confirmed the existence of a large array of hemorheological abnormalities in SLE and RA and provided

Table 4
Variables associated with cIMT above the median.

	Univariate analysis* OR (95%CI)	Domain-specific multivariate analysis† OR (95%CI)	Final model OR (95%CI)
Demographics			
Age	1.15 (1.09;1.21)		
Education	0.88 (0.81;0.96)		
Menopause	29.17 (10.60;80.24)	28.57 (10.38;78.67)	34.72 (4.44;271.77)
Disease characteristics			
Age at disease diagnosis	1.11 (1.06;1.19)	1.13 (1.08;1.18)	
Disease duration	1.06 (0.99;1.13)	1.14 (1.05;1.23)	
Hematocrit	1.09 (0.99;1.19)		
Traditional CV risk factors			
Hypertension	4.09 (1.89;8.84)	3.09 (1.37;6.98)	
Dyslipidemia	2.22 (1.02;4.83)		
Obesity	3.40 (1.54;7.51)	2.39 (1.02;5.57)	4.09 (1.00;16.68)
Waist circumference	1.04 (1.00;1.07)		
Current medication			
Corticosteroid dose	0.91 (0.85;0.98)	0.91 (0.84;0.99)	0.87 (0.78;0.98)
MTX use	2.13 (1.02;4.44)		
APS use	0.38 (0.18;0.80)	0.37 (0.17;0.81)	
Hemorheological parameters			
WBVn 22.5 s^{-1}	2.30 (1.16;4.56)	2.82 (1.37;5.79)	3.98 (1.23;12.83)
ED6	1.10 (1.00;1.21)	1.10 (1.00;1.21)	
NO	0.55 (0.30;1.00)	0.47 (0.24;0.89)	0.16 (0.05;0.52)

Results are expressed as odds ratios (OR) and 95% confidence intervals (CI).
* Variables identified in univariate analysis at a $p \leq 0.10$.
† Variables identified in domain-specific models at a $p < 0.05$.

evidence that both inflammation and traditional CV risk factors are related to altered rheological parameters of blood.

The rational to conduct a study in SLE and RA patients was based on the similarities between these two pathologies – both are systemic rheumatic inflammatory diseases associated with an increased risk of premature atherosclerosis – and on the assumption of a common mechanism underlying accelerated atherogenesis. In view of the controversy regarding the temporal relationship between hemorheological changes and the thrombotic event [17] only individuals without previous CV events were included.

Patients presented decreased whole blood viscosity as well as lower erythrocyte deformability and increased erythrocyte aggregation as compared to the non-inflammatory controls. Reduced ED together with increased EA may contribute to impaired microcirculation and add to microvascular dysfunction [18–20], a complication that was documented in SLE and RA patients even in the absence of macrovascular disease [21]. We found ED to be correlated with CV risk factors and to be reduced in the presence of metabolic syndrome. However, no relationship with inflammatory parameters could be depicted neither a significant difference between remission and active disease was evident, suggesting that altered deformability is a more stable phenomenon not influenced by current levels of inflammation in this group of patients. Furthermore, in multivariate analysis ED was not a significant predictor of subclinical atherosclerosis.

Red cell disaggregation is considered fundamental for the fluidity of blood, especially in low-shear regions of the circulatory system. Increased EA found in association with LAC, RF and/or anti-CCP antibodies and in the presence of metabolic syndrome may therefore have implications for atherothrombosis in this high risk population. Additionally, in the presence of metabolic syndrome, the increase of EA was more pronounced in patients with active disease, suggesting a potentiating effect between these two conditions.

Decreased whole blood viscosity in SLE and RA patients is in contrasts with some previous reports [12–14]. WBV is a global rheological parameter that largely depends on hematocrit, erythrocyte deformability and plasma viscosity, being fibrinogen one of the principal determinants of this one [13,22]. SLE and RA women had lower hematocrit than controls and WBV highly correlated with hematocrit levels. In favor of this explanation is also the finding that in active disease WBV decreases in parallel to hematocrit. Also Vaya and collaborators reported lower WBV in SLE patients in relation with diminished hematocrit levels [23]. Moreover, we cannot exclude that sample size issues and patient heterogeneity might have contributed to the different results, as some of the previous publications included small numbers and clinically more heterogeneous patient populations.

Subclinical atherosclerosis in SLE and RA patients was associated with some hemorheological parameters. We documented a bivariate association between atherosclerotic plaques and increased EA and reduced erythrocyte NO, but the small number of patients with plaques hampered further analysis and limited the interpretation of these findings. On the other hand, increased WBV and reduced NO retained significant associations with cIMT above the median, after controlling for other covariates and confounders in multivariate analysis. In the general population, increased blood viscosity was identified in association with CV risk factors such as gender, age, hypertension, diabetes, metabolic syndrome [24] and also with the risk of CV events [8], but little is known with regard to rheumatic diseases. Booth et al reported high WBV in a subgroup or SLE patients with prevalent atherothrombotic events, although in the whole group of patients, blood viscosity did not differ from controls [25]. To the best of our knowledge, the present work shows for the first time an independent association between WBV and cIMT in young women with inflammatory rheumatic diseases. This positive association between WBV and cIMT is in line with previous observations in male subjects without inflammatory diseases [26]. Yet the role of WBV in the pathophysiology of atherosclerosis is not entirely elucidated; this is a striking finding in patients in whom the average WBV is lower than in control subjects. Even though atherosclerosis is recognized as an inflammatory disease, lesions do not occur randomly, but affect preferentially sites where blood flow is disturbed, highlighting the role of the shear forces [27]. Blood viscosity may account to the development of atherosclerosis through a mechanical effect on the arterial wall, contributing to changes in endothelial cells that promote inflammation [28]. On the other hand, endothelial cells in SLE and RA already display an activated phenotype due to chronic inflammation which increase their susceptibility to atherosclerosis [29]. Thus, in the presence of chronic inflammation, even lower WBV might be harmful. Even so, reduced

WBC is blunted in the presence of metabolic syndrome, putting in evidence the interplay between inflammation, traditional CV risk factors and rheological characteristics of blood.

We could not find any significant differences in erythrocyte NO production in relation to diagnosis, disease activity or metabolic syndrome. Surprisingly, NO was significantly associated with both cIMT and presence of plaques (negative association) and was an independent predictor of cIMT. Erythrocyte NO production can be looked at as a compensatory mechanism. Under low oxygen tension, erythrocytes release NO bound to hemoglobin, promoting vasodilation [30]. Besides its effects on vascular resistance, through antithrombotic, anti-inflammatory and anti-proliferative effects NO could represent a protective factor against atherosclerosis. However, NO plays a dichotomous role and excessive and prolonged production of NO may contribute to tissue damage. The role of NO in chronic inflammation is complex and deserves further clarification in SLE and RA.

Even though SLE and RA share similarities, there are some differences in hemorheological parameters between these two patient groups. Compared to RA, women with SLE present a less favorable hemorheological profile, characterized by higher blood viscosity and increased concentrations of fibrinogen, a parameter associated with greater risk of arterial thrombosis as well as with higher mortality in the general population [31]. The increase in erythrocyte aggregation is similar in SLE and RA, regardless of antimalarial and aspirin medication being more often prescribed to lupus patients. We hypothesize that in addition to the presence of LAC, higher levels of fibrinogen in SLE patients may contribute to aspirin resistance, in agreement with what was previously observed in other populations [32].

In conclusion, the present study shows that hemorheological parameters are independently associated with the early stages of atherosclerosis in SLE and RA patients. Additionally, it documents disturbed and unfavorable hemorheological features in association with disease activity and with traditional CV risk factors. Therefore, abnormal rheological characteristics of blood might represent an additional mechanism that contributes to atherogenesis in inflammatory rheumatic diseases.

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PARTE V

Different patterns of early vascular alterations in SLE and RA patients - a step towards understanding the associated cardiovascular risk

Different patterns of early vascular alterations in SLE and RA patients - a step towards understanding the associated cardiovascular risk

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Abstract

Objective - To compare vascular biomarkers and endothelial function between women with systemic lupus erythematosus (SLE) and those with rheumatoid arthritis (RA) without previous cardiovascular (CV) events.

Methods - Female SLE (n=127) and RA (n=107) patients and a control group of 124 women without inflammatory diseases were studied. Circulating levels of intercellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (sVCAM-1), thrombomodulin (TM), and tissue factor (TF) were measured and endothelial function was assessed using peripheral artery tonometry. Reactive hyperemia index (RHI), an indicator of microvascular reactivity, and augmentation index (AIx), a measure of arterial stiffness, were obtained. In addition, traditional CV risk factors, disease activity and markers of inflammation (fibrinogen, C-reactive protein, tumor necrosis factor, interleukin-18, monocyte chemoattractant protein-1, and macrophage migration inhibitory factor (MIF)) were determined.

Results - Women with SLE displayed higher sICAM-1 and TM and lower TF levels than RA women ($p=0.001$, $p<0.001$ and $p<0.001$, respectively). These differences remained significant after controlling for CV risk factors and medication and were more pronounced in active disease. Although RHI was similar across the groups, AIx was increased in lupus as compared to RA ($p=0.04$). Also in active SLE, a trend towards poorer vasodilation was observed ($p=0.06$). Disease activity, fibrinogen and MIF levels were independently associated with endothelial dysfunction.

Conclusion - SLE and RA women present with distinct patterns of endothelial activation biomarkers, and these are not explained by differences in traditional CV risk factors. Early vascular alterations are more pronounced in SLE and particularly when the disease is active.

Key messages:

- Early vascular changes are more pronounced in women with SLE than in those with RA
- SLE and RA women show distinct patterns of endothelial activation biomarkers, particularly in active disease
- Disease activity is independently associated with endothelial dysfunction

Chronic systemic inflammation predisposes to accelerated atherosclerosis, a risk that is well known in systemic lupus erythematosus (SLE) and in rheumatoid arthritis (RA) patients [1]. Subclinical vascular lesions develop long before atherosclerosis becomes clinically evident, and they progress more rapidly in SLE and RA than in the general population [2, 3]. Traditional cardiovascular (CV) risk factors do not fully explain this enhanced risk, and the disease itself is considered an independent CV risk factor [1]. However, the reported magnitude of the CV risk is several times higher in SLE than in RA, and the reason for this divergence is still incompletely understood [4-7].

Endothelial damage is considered the first step in the pathogenesis of atherosclerosis [8]. The importance of endothelial cells (ECs) for vascular health is highlighted by its crucial role in maintaining blood fluidity and regulating the vascular tonus and permeability. Under basal conditions ECs express molecules such as thrombomodulin (TM), which prevent platelet aggregation and the activation of the clotting cascade. Further platelet inhibition is achieved as a result of nitric oxide (NO) synthesis, a major vascular relaxant with anti-inflammatory and anti-proliferative properties. During the inflammatory process, ECs undergo changes characterized by enhanced expression of adhesion molecules, increased transendothelial permeability, and loss of antithrombotic properties [9]. Pro-inflammatory cytokines suppress TM expression and promote its cleavage and release into circulation [10]. In addition, they induce the expression of tissue factor (TF), a procoagulant molecule absent from the surface of the intact ECs [11], shifting the balance towards a prothrombotic state. Furthermore, damaged endothelium loses its ability to produce vasodilators, thus adding to the vascular injury.

Given the clinical and physiopathological particularities of SLE and RA, we hypothesize that endothelial function is differently disturbed in these two patient groups,

which could explain the different CV risk. Thus, the major aim of our study was to compare endothelial cell function between SLE and RA as assessed by the measurement of soluble vascular biomarkers and by endothelial function testing, taking into account the presence of traditional CV risk factors and systemic inflammation. Subsequently we investigated the variables associated with endothelial dysfunction in SLE and RA patients.

Methods

Subjects

Consecutive SLE and RA women fulfilling the ACR classification criteria and free of clinically manifest CV disease were recruited from the rheumatology clinics of Hospital Garcia de Orta, Almada, and Hospital de Santa Maria, Lisbon, between April 2009 and October 2010. A control group of women without systemic inflammatory disease was also recruited from the local community and evaluated in the same period. Participants were excluded if they were pregnant, breastfeeding, had impaired renal function (defined as serum creatinine>1.5 mg/dl), or had documented ischemic heart disease (previous infarction, revascularization surgery, angina, or heart failure), cerebrovascular disease (stroke or transient ischemic attack) or symptomatic peripheral artery disease. The study was approved by the Ethics Committee of both hospitals and was conducted in accordance with the principles stated in the Declaration of Helsinki. All participants gave written informed consent.

Clinical assessment

Clinical assessment included demographic data, disease features, medication, and CV risk profile: age, blood pressure, serum lipids, fasting glycemia, smoking habits, and body mass index (BMI). Patients were diagnosed with hypertension if the measured blood

pressure was repeatedly ≥140/90 mm/Hg or if they used antihypertensive medication. The diagnosis of diabetes was made is fasting glucose level was ≥126 mg/dl, or if patients were under pharmacologic treatment. Participants were classified as obese if BMI was ≥30Kg/m^2. Disease activity was evaluated using the SLE Disease Activity Index 2000 (SLEDAI 2K), [12] and in RA patients 28 joints were examined for tenderness and swelling, and the 4 variable disease activity score (DAS28) was calculated using erythrocyte sedimentation rate [13]. Disease activity was stratified into 3 categories according to the cutoffs of each instrument [13, 14]: remission (SLEDAI 2K = 0 for SLE or DAS28 <2.6 for RA patients), low disease activity (≥1 SLEDAI 2K <4, in the case of SLE, or ≥2.6 DAS28 <3.2, in the case of RA), and active disease (SLEDAI 2K ≥4 or DAS28≥3.2, for SLE and RA patients, respectively).

Fasting blood samples were collected for measurement of glucose, uric acid, lipids (total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and triglycerides), inflammatory mediators (C-reactive protein (CRP), fibrinogen, tumor necrosis factor (TNF), interleukin (IL)-18, monocyte chemoattractant protein (MCP) -1 and macrophage migration inhibitory factor (MIF)) and soluble vascular biomarkers (soluble intercellular adhesion molecule (sICAM)-1, soluble vascular cell adhesion protein (sVCAM) -1, thrombomodulin, and tissue factor).

Quantification of soluble vascular biomarkers and cytokines

Measurements were performed using commercial enzyme-linked immunosorbent assay (ELISA) based methods according to the manufacturers' instructions. The Human sICAM-1 FlowCytomix Simplex Kit and the Human sVCAM-1 FlowCytomix Simplex Kit (Bender MedSystems GmbH, Vienna, Austria) were used for quantification of adhesion molecules, both using the FlowCytomix ™ Technology. Serum levels of TM were measured using the

Human Thrombomodulin ELISA Kit (Cell Sciences ®, Canton, MA, USA) and serum levels of TF were quantified using the AssayMax Human Tissue Factor ELISA kit (Assaypro, St Charles, Mo, USA). The cytokines TNF, IL-18, and MCP-1 were analyzed using the FlowCytomix Kits (Bender MedSystems GmbH, Vienna, Austria) and serum MIF levels were quantified using the AssayPro Human MIF ELISA Kit (St Charles, Mo, USA).

Endothelial function tests

Endothelial function was assessed by peripheral artery tonometry (PAT) in a subgroup of 87 women with SLE, 75 with RA, and 83 controls with demographic and clinical characteristics comparable to the entire study population. PAT is a noninvasive method that evaluates changes in pulse wave amplitude before and after reactive hyperemia. The exam was performed using the EndoPAT 2000 device (Itamar Medical Ltd, Cesarea, Israel) as described elsewhere [15] and by assessors blinded to the clinical diagnosis. Briefly, patients were placed in a quiet room, in supine position, with a specially designed finger probe on the index finger of each hand, and a pressure cuff placed on one arm. Patients were recommended to refrain from smoking and drinking coffee or tea during the previous 24 hours and not to eat for at least 6 hours preceding the exam. PAT was continuously measured during a 10-minute baseline period, for 5 minutes after the pressure cuff was inflated to suprasystolic pressure and for 10 additional minutes following the release of upper arm occlusion. Reactive hyperemia index (RHI) was calculated as the ratio of PAT signal amplitude after cuff deflation divided by the amplitude of baseline signal, adjusted for fluctuations in the magnitude of the signal in the contralateral finger. Endothelial dysfunction (ED) was defined as a RHI ≤1.67, according to manufacturer specifications. Augmentation index (AIx) was calculated from the mean PAT waveform of the baseline

period dividing the amplitude of the second systolic peak by the difference between the second and the first peak.

Statistical analysis

Continuous variables are expressed as means with standard deviations and categorical variables as the number of affected individuals and proportion of the total. Demographic data, traditional CV risk factors, and medication were compared across SLE, RA and controls using independent Anova, Kruskal-Wallis or χ^2 tests, followed by correction for multiple comparisons, as appropriate. Spearman's coefficients were calculated to establish the correlation between vascular biomarkers and inflammatory parameters.

Vascular biomarkers, as well as RHI and AIx, were compared between SLE and RA patients first as crude means using the Mann Whitney test, and subsequently using general linear models and incorporating as covariates the baseline variables that were significantly different between patients' groups. After that, the effect of disease activity on the studied outcomes was examined by comparing remission with active disease.

In order to assess the independent association of vascular biomarkers, inflammatory parameters, traditional CV risk factors, and medication with endothelial dysfunction, age adjusted univariable logistic regression analyses were undertaken and those variables associated with the study outcome with a p value ≤ 0.15 were subsequently entered in a multivariable logistic regression model (backwards selection). Removed variables were re-entered into the final model to check for possible confounding.

Statistical analysis was performed assuming a 5% significance level and using SPSS 17 for Windows.

Results

In total 127 women with SLE, 107 with RA, and 124 female controls were included in the study. Demographic and clinical characteristics of the participants are shown in Table I. SLE women were younger and had shorter disease duration (8.4±6.5 years) compared to women with RA (10.7±7.3 years, p=0.01). All lupus patients were ANA positive and 88.8% of RA patients were positive either for IgM RF or for anti-citrullinated protein antibodies. The use of antimalarials and aspirin was more common in lupus, while more RA patients received methotrexate. Patients presented a broad range of disease activity. The mean SLEDAI 2K was 3.46 ±4.5 (range 0 to 21) and the mean DAS28 was 4.19±1.4 (range 1.70 to 7.54). Disease was in remission in 40.9% of SLE and in 16.8% of RA cases. 38.6% of SLE patients presented active disease defined as a SLEDAI 2K≥4, and 72% of RA had active disease according to the DAS28 definition. Serum concentrations of CRP, IL-18, MCP-1, and MIF were comparable in both patient groups, but fibrinogen was higher in SLE (SLE 326±147 mg/dl vs RA 276±101 mg/dl; p=0.02), while serum TNF was higher in RA (SLE 10.4±47 pg/ml vs RA 13.7±30 pg/ml; p<0.001).

Vascular biomarkers and endothelial function as assessed by PAT

Concentrations of circulating vascular biomarkers and results of endothelial function from SLE and RA patients are shown in Figure 1. Results from the control group are also presented as reference. A distinct pattern of soluble ECs biomarkers was identified in SLE and in RA. While sICAM-1 and TM levels were significantly higher, TF was lower in lupus than in RA patients (Figure 1). Differences in sICAM, TM and TF remained significant after adjustment for age, disease duration, medication, and lipid levels (Table II).

On the whole, PAT results were similar in patients and controls. RHI shown a significant decrease with disease duration (ß per quartile of disease duration = -0.203, 95%CI

-0.235 to -0.033; p=0.009). Reduced RHI, indicating endothelial dysfunction (ED), was found in 27 (31 %) women with lupus and in 20 (26.7 %) with RA (p=0.54). AIx was significantly higher in SLE as compared with RA (p=0.04), indicating increased arterial wall stiffness in these patients. This increase remained statistically significant after controlling for differences in baseline characteristics (p=0.005) (Table II).

Disease activity, vascular biomarkers and endothelial function

Among patients, biomarkers of endothelial activation shown a weak to moderate positive correlation with several inflammatory parameters: sICAM-1 correlated with serum TNF (rho 0.196), IL-18 (rho 0.249), MCP-1 (rho 0.327), and MIF (rho 0.244) (p<0.001); sVCAM-1 correlated with IL-18 (rho 0.275) and MCP-1 (rho 0.266) (p<0.001); TM correlated with MIF (rho 0.305, p<0.001) and TF correlated with TNF (rho 0.222) and C-reactive protein (rho 0.225) (p=0.001).

The mean values of sICAM-1, sVCAM-1, and TF were higher in active disease than in remission (p=0.001; p=0.01 and p<0.001, respectively). When in remission, serum levels of endothelial biomarkers in SLE women were not much different from RA women. However, in active disease, the increase in sICAM-1, sVCAM-1 and TM was greater in lupus than in RA patients. (3936±1803 ng/ml vs 598±270 ng/ml, p=0.003; 1982±320 ng/ml vs 1183±400 ng/ml, p=0.01; 6.98±3.4ng/ml vs 5.01±2.7ng/ml, p=0.01, respectively). On the other hand, patients with active RA displayed higher serum TF compared with patients with active SLE (187±14.7 pg/ml vs 63±69 pg/ml, p<0.001). The differences in sVCAM-1, TM and TF remained significant after controlling for differences in baseline characteristics (Table III).

Patients' RHI and AIx did not differ from controls. RHI was lower in lupus than in RA patients, either in active disease or in remission, but the difference did not reach statistical significance. Lupus patients presented increased AIx compared with RA patients regardless

of the disease activity status - in remission 15.5 vs 8.5, p=0.04; in active disease 18.3 vs 9.9, p=0.03. After controlling for covariates, the differences in AIx related to disease activity status were no longer significant (Table III).

Endothelial dysfunction was more prevalent in patients with active disease (33.7%) than in remission (17%), (p=0.03). In multivariate logistic regression adjusted for age, the variables associated with ED in women with SLE and RA were lower education level, active disease, higher MIF, higher serum uric acid, higher fibrinogen levels and lower LDL cholesterol (Table IV). Circulating levels of vascular biomarkers were not independently associated with ED.

Discussion

In this comparative study we found distinct patterns of soluble vascular biomarkers in SLE and in RA female patients free from clinically evident CV disease. Lupus patients presented higher serum sICAM-1 and TM levels, while TF was elevated in RA patients. These findings are relevant for understanding the physiopathology of the increased CV risk in SLE and RA patients, as cell adhesion molecules may represent a link between inflammation and atherosclerosis. In fact, not only are VCAM-1 and ICAM-1 highly expressed on the endothelium overlaying atherosclerotic lesions [16, 17], but an increased serum concentration of these molecules is also related to CV risk factors [18] and incident myocardial infarction [19]. In particular, high serum levels of ICAM-1 represent an independent risk factor for atherosclerosis and a predictor of future CV events [19, 20]. In addition, we observed a significant correlation between inflammatory markers (TNF, IL-18, MIF, and MCP-1) and soluble cell adhesion molecules, and also increased sICAM-1 and sVCAM-1 levels in active disease. These observations are in line with previous studies demonstrating that inflammatory mediators, including TNF, IL-6, interferon-gamma (INFγ)

[21], IL-18 [22], but also MCP-1 and MIF [23], upregulate endothelial cell adhesion molecule expression. The fact that SLE patients exhibit higher sICAM-1 and also higher fibrinogen concentrations may be relevant in the initiation and progression of atherosclerosis. Indeed, ICAM-1 serves as a binding site for fibrinogen and promotes adhesion and transendothelial migration of leukocytes [24], an important early step in inflammatory vascular disease. We did not find any difference in VCAM-1 serum levels among the studied groups. In animal models, VCAM-1 expression is considered a major early event in the atherosclerotic process [25], and increased sVCAM-1 levels have been reported in lupus nephritis [26]. However, in RA and SLE patients without renal or vascular disease, serum concentrations of VCAM-1 are similar to the control population and the relationship to atherosclerosis is uncertain [27, 28].

There is growing evidence supporting the relationship between inflammation and thrombotic complications of atherosclerosis (atherothrombosis). Interestingly, TM expression, a molecule with anti-coagulant properties, is reduced during the inflammatory process [10], and increased soluble TM levels probably indicate EC injury [29]. Together with increased TF, which is an initiator of the extrinsic coagulation cascade, this environment may raise the thrombogenic activity of plasma and contribute to cardiovascular events. Higher levels of TF in RA patients as compared to SLE patients might be explained by the contribution of TNF to its expression [30]. Nevertheless, serum levels of adhesion molecules, TM, and TF may not accurately translate endothelial functional expression of these molecules, which is a limitation of our work.

A further effect of proinflammatory cytokines on EC is the inhibition of NO synthesis leading to endothelial dysfunction. In the general population, impaired endothelial function is a critical early step in the development of atherosclerosis [31] and predicts the progression of structural arterial disease independently of conventional CV risk factors [32, 33].

However, studies of endothelial function in inflammatory rheumatic diseases depicted contradictory results [34-36], and the relevance of endothelial dysfunction for the progression of atherosclerosis in rheumatic diseases remains uncertain [37, 38]. Using PAT, we did not find any significant differences in RHI neither between patients and controls, nor between SLE and RA. Moreover, the proportion of ED was also similar across the 3 groups (data not shown). Nevertheless, RHI significantly decreased as a function of disease duration, and the odds of ED were 3 times higher in active disease as compared to remission. Furthermore, even mild disease activity was independently associated with ED. RHI quantifies changes in pulse wave amplitude in response to reactive hyperemia, a measure of microvascular function. In the general population RHI is an independent predictor of adverse cardiac events [39], but its predictive value in rheumatic diseases has not been established. The fact that we have included only females without previous CV events and normal renal function (relatively low risk population) may in part account for the comparable RHI found in patients and controls. In fact, only in more active and long-lasting disease cases did RHI show significant reduction. The follow up of these patients will allow us to ascertain the predictive value of ED measured by PAT for the development of CV event in SLE and RA patients.

Lupus patients presented higher AIx than RA patients and this difference remained significant after controlling for covariates. AIx is a measure of arterial stiffness and has been associated with cardiovascular risk and target organ damage in the general population [40]. Little is known about AIx in SLE and RA. Shang et al found a correlation between carotid AIx and SLEDAI [41], and increased arterial stiffness was demonstrated in long-standing RA [42]. Cardiovascular risk factors and disease related features contribute to arterial stiffening in SLE [43, 44] and RA [45]. Increased AIx in SLE women probably indicates a worse vascular condition.

Taken together, our observations add to the evidence that pathogenesis of atherosclerosis associated with inflammation may differ in SLE and RA. Additionally, we found more pronounced early vascular changes in lupus patients, and when the disease is active, which is in line with the higher risk for CV events documented in these patients.

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Conflicts of interest with regard to the present work: none

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Table I - Demographic and clinical characteristics of SLE, RA and control women

	SLE (n=127)	RA (n=107)	Controls (n=124)	p value
Demographic data				
Age, years	43.9 (13.9) [*1]	50.2 (14.1)	46.9 (13.7)	0.003
Education, years	8.9 (4.8)	8.3 (5.2)	9.4 (5.2)	0.30
Caucasians, n (%)	110 (86.6)	95 (88.8)	121 (97.6) [†1]	0.006
Menopause, n (%)	60 (47.2)	62 (57.9)	64 (51.6)	0.25
Traditional CV risk factors				
Current smoker, n (%)	18 (14.2)	17 (15.9)	25 (20.2)	0.39
Hypertension, n (%)	51 (40.2)	37 (34.6)	31 (25.0) [†2]	0.04
Total cholesterol, mg/dl	189.3 (42.8) [*2]	204.0 (33.5)	204.6 (33.9)	0.002
HDL cholesterol, mg/dl	56.6 (16.1) [*3]	63.9 (18.5)	62.1 (14.9)	0.003
LDL cholesterol, mg/dl	113.9 (36.6)	123.4 (28.5)	126.5 (30.2) [†3]	0.01
Triglycerides, mg/dl	125.7 (88.2) [*2]	103.1 (42.8)	97.6 (42.1)	0.02
Diabetes, n (%)	9 (7.1)	5 (4.7)	8 (6.4)	0.73
Obesity, n (%)	33 (25.9)	33 (30.8)	32 (25.8)	0.69
Medication				
Antihypertensive, n (%)	51 (40.2)	32 (29.9)	28 (22.6) [†4]	0.01
Lipid lowering, n (%)	31 (24.4)	17 (15.9)	21 (16.9)	0.18
Aspirin, n (%)	28 (22)* [4]	6 (5.6)	0 (0)	<0.001
Antimalarials, n (%)	97 (74) [*5]	20 (18.7)	-	
Methotrexate, n (%)	12 (9.5) [*5]	85 (82.5)	-	
Prednisolone, mg/day	7.8 (10.9) [*5]	3.2 (3.5)		

Results are presented as means (SD) or number of affected individuals and (%).

* statistically significant differences between SLE and RA : [*1]p=0.002; [*2]p=0.01; [*3]p=0.007; [*4]p=0.001; [*5]p<0.001

† statistically significant differences between the control group and either SLE or RA: [†1]p=0.002 vs SLE and p=0.01 vs RA; [†2]p=0.02 vs SLE; [†3]p=0.01 vs SLE; [†4] p=0.005 vs SLE;

SLE - systemic lupus erythematosus; RA – rheumatoid arthritis; BP - blood pressure; HDL - high density lipoprotein; LDL - low density lipoprotein.

Table II - Vascular biomarkers and results of PAT assessment in SLE and RA, after controlling for baseline characteristics

	SLE (n=127)	RA (n=107)	p value
sICAM, ng/ml	2057(876)	334(895)	0.05
sVCAM, ng/ml	1407(162)	1080(167)	0.24
TM, ng/ml	6.50(0.36)	4.55(0.37)	0.002
TF, pg/ml	49.3 14.4)	158(14.7)	<0.001
RHI*	2.022(0.11)	2.29(0.12)	0.16
AIx*	20.4 (2.4)	8.1 (2.7)	0.005

Results are presented as estimated marginal means (SE) adjusted for differences in baseline characteristics (age, total cholesterol, HDL, LDL, disease duration, aspirin, hydroxichloroquine and methotrexate use, and prednisolone dose)

*RHI and AIx results refer to 87 women with SLE, 75 with RA and 83 controls

sICAM-1 - soluble intercellular adhesion molecule; sVCAM-1 - soluble vascular cell adhesion protein; TM – thrombomodulin; TF – tissue factor; RHI - reactive hyperemia index; AIx - augmentation index.

Table III - Vascular biomarkers and endothelial function in remission and active disease, after correction for baseline characteristics

	Remission			Active disease		
	SLE (n=127)	**RA (n=107)**	p value	**SLE (n=127)**	**RA (n=107)**	p value
sICAM-1	1836 (1421)	332(2174)	0.59	3032 (1544)	382 (1147)	0.23
sVCAM-1	1107 (256)	1023 (393)	0.87	2117 (379)	1041 (224)	0.04
TM	6.02 (0.53)	4.50 (0.81)	0.62	6.48 (0.58)	4.64 (0.43)	0.03
TF	36.1 (23.2)	95.4 (32.7)	0.18	48.7 (21.4)	175 (17.3)	<0.001
RHI*	2.237 (0.15)	2.315 (0.22)	0.79	1.806 (0.16)	2.242 (0.13)	0.06
AIx*	17.8 (3.4)	8.5 (0.5)	0.06	17.2 (3.6)	8.0 (2.9)	0.07

Results are expressed as estimated marginal means (SE) adjusted for differences in baseline characteristics (age, total cholesterol, HDL, LDL, disease duration, aspirin, hydroxichloroquine and methotrexate use, and prednisolone dose)

*RHI and AIx results refer to 87 women with SLE, 75 with RA and 83 controls

SLE - systemic lupus erythematosus; RA – rheumatoid arthritis; sICAM-1 - soluble inter-cellular adhesion molecule; sVCAM-1 - soluble vascular cell adhesion protein; TM – thrombomodulin; TF – tissue factor; RHI - reactive hyperemia index; AIx - augmentation index.

Table IV - Variables associated with endothelial dysfunction in systemic lupus erytematosus and rheumatoid arthritis female patients

	Univariate analysis		**Multivariate analysis**	
	OR (95%CI)	p value	**OR (95%CI)**	p value
Education, years	0.934 (0.869-1.003)	0.06	0.853 (0.763-0.966)	0.006
MIF, pg/ml	1.000 (1.000-1.000)	0.08	1.000 (1.000-1.000)	0.01
Fibrinogen, mg/dl	1.003 (1.000-1.006)	0.08	1.005 (1.001-1.009)	0.02
Uric acid, mg/dl	1.306 (0.987-1.729)	0.06	1.398 (1.000-1.955)	0.05
LDL cholesterol, mg/dl	0.991 (0.980-1.002)	0.10	0.987 (0.974-1.000)	0.04
Triglycerides, mg/dl	1.003 (0.999-1.008	0.12	ns	
Current steroids vs no steroids	1.697 (0.849-3.393)	0.13	ns	
Low activity vs remission	3.056 (1.051-8.881)	0.04	3.898 (1.071 -14.191)	0.04
Active disease vs remission	2.489 (1.065-5.819)	0.03	3.009 (1.045-8.662)	0.04

MIF - macrophage migration inhibitory factor; LDL - low density lipoprotein

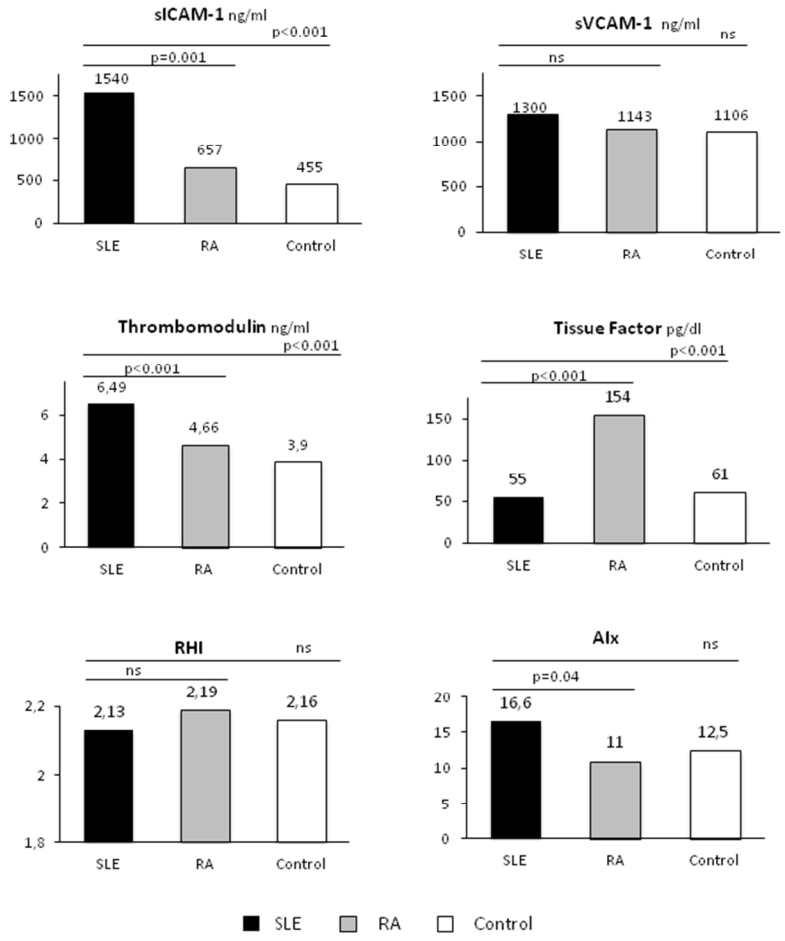

Figure 1: Serum concentrations of vascular biomarkers and endothelial function tests in SLE and RA patients and non-inflammatory controls. The groups were compared using the Kruskal-Wallis test (on the upper line is displayed the p value across the groups) and the post-hoc Dunett T3 was used for multiple comparisons (significant differences between SLE and RA are shown on the lower lines).
sICAM-1 - soluble intercellular adhesion molecule; sVCAM-1 - soluble vascular cell adhesion protein; RHI - reactive hyperemia index; AIx - augmentation index; SLE - systemic lupus erythematosus; RA – rheumatoid arthritis.
190x254mm (96 x 96 DPI)

DISCUSSÃO

Nos últimos anos, a comunidade científica dedicou particular atenção à patologia CV que acompanha as doenças reumáticas inflamatórias sistémicas. A investigação epidemiológica demonstrou de forma inequívoca o importante contributo dos eventos CV para a morbilidade e mortalidade associada a estas doenças, constituindo uma das principais causas de morte dos doentes com LES ou com AR. Apesar dos grandes avanços no tratamento destas doenças reumáticas, a mortalidade CV manteve-se inalterada ao longo das últimas décadas, ao invés do decréscimo nas mortes CV que se observou na população dos países ocidentais.

A investigação clínica e básica, visando a melhor compreensão dos intervenientes nas várias etapas do processo aterosclerótico associado às doenças reumáticas, traduziu-se num número crescente de publicações dedicadas a esta problemática nos últimos anos. Sabemos que os factores de risco tradicionais e a inflamação concorrem para a aterosclerose precoce e acelerada, mas o contributo relativo de cada um destes intervenientes, assim como a sua interacção, permanecem por esclarecer. Se atendermos a que o LES e a AR são ambas doenças inflamatórias mas com um risco CV diferente, o estudo comparativo destas doenças pode identificar processos e elementos chave na aterogénese.

Embora não explicando totalmente o risco CV acrescido, os factores de risco tradicionais contribuem de forma substancial para o risco CV no LES e na AR [73, 123, 250]. O reconhecimento da importância dos FR levou a que a EULAR emanasse recomendações nesta área [251, 252]. Contudo, a sensibilização dos clínicos para o rastreio sistemático e adopção de medidas preventivas nos doentes reumáticos é ainda reduzida [253]. Pudemos confirmar a dimensão deste problema numa amostra de reumatologistas portugueses, o que significa que existe um vasto campo para implementar melhores cuidados preventivos [254].

Com o intuito de caracterizar a prevalência de factores de risco CV tradicionais nestas duas doenças, aplicámos numa primeira fase um protocolo de avaliação a doentes do sexo feminino sem eventos CV prévios. Nesta primeira parte do trabalho confirmámos que perto de 80% das doentes apresentam pelo menos um FR CV de Framingham, e que a HTA é o factor modificável mais prevalente (Parte I). A probabilidade de HTA é cerca de duas vezes superior entre as doentes do que entre os controlos do mesmo sexo e idade sem doença inflamatória. Para esta diferença contribuem de forma substancial as doentes mais jovens (idade <40 anos), onde a HTA é significativamente mais frequente. A HTA, juntamente com a insulino-resistência e obesidade central, também mais comuns no grupo de doentes, explica a prevalência mais elevada de síndrome metabólica no LES (27%) e na AR (25,5%) do que nos controlos (15,7%). Ainda que nenhuma doente tivesse valores de uricémia para além do limite superior do normal, este grupo de doentes com função renal preservada apresenta um valor médio de ácido úrico superior ao dos controlos. Diversos trabalhos identificaram a uricémia como um FR independente para doença CV na população geral, particularmente entre indivíduos hipertensos [255, 256], e o benefício CV de alguns anti-hipertensores foi em parte atribuído ao seu efeito na redução dos níveis de ácido úrico [257]. Os valores mais elevados de ácido úrico entre as mulheres com LES ou com AR poderá contribuir para o acréscimo do risco vascular.

Ainda no que respeita aos FR CV encontrámos algumas diferenças entre as mulheres com LES e as mulheres com AR. A HTA é mais frequente nas mulheres jovens com LES, enquanto nas mulheres com AR prevalece a insulino-resistência. Foi igualmente na AR que identificámos modificações da composição corporal em que predomina o aumento da massa gorda (Parte II). Este excesso de adiposidade observou-se quer nas doentes com peso normal, quer nas que têm

excesso de peso ou obesidade, de acordo com as categorias do BMI. Para além do aumento da quantidade, as mulheres com AR apresentam também modificações na distribuição da massa gorda. A obesidade central, com implicações conhecidas no acréscimo do risco vascular [258], está presente em mais de 3/4 destas mulheres. A sarcopénia, embora menos estudada na relação com eventos CV, é um preditor de pior qualidade de vida e de mortalidade global [259]. Apenas no grupo de doentes identificámos sarcopénia, quer isoladamente, quer em associação com excesso de massa gorda, originando o fenótipo conhecido por obesidade sarcopénica e que alguns trabalhos associaram ao aumento da rigidez da parede arterial em indivíduos sem doença vascular conhecida [260].

No que respeita às alterações quantitativas dos lípidos, também são notórias as diferenças entre o LES e a AR. Nas doentes com LES encontrámos valores diminuídos do colesterol total e das suas fracções LDL e HDL. No entanto, ao analisarmos a relação Col/HDL e o índice aterogénico do plasma, foi este grupo que apresentou um perfil lipídico mais aterogénico.
As características da doença, entre as quais se inclui a actividade inflamatória, bem como alguns fármacos utilizados no seu tratamento, explicam em parte as alterações observadas no perfil dos factores de risco nas doentes com LES e nas doentes com AR.

Neste mesmo grupo de mulheres explorámos a hipótese de polimorfismos de genes que codificam citocinas chave no processo inflamatório e que foram associados a risco aumentado de eventos CV, serem mais prevalentes no LES e na AR do que entre os controlos. Estudámos os polimorfismos do promotor do gene do TNF na posição -308 G>A, do promotor da IL6 na posição -174 G>C e da linfotoxina A na posição 252 A>G que, apesar de alguma controvérsia, têm implicações funcionais [261-263]. Ao condicionarem os níveis de transcrição dos genes, poderão afectar a susceptibilidade para a

doença reumática e influenciar a sua expressão fenotípica assim como o risco CV associado. Os resultados preliminares da avaliação destes polimorfismos confirmaram a associação com algumas manifestações clínicas do LES [264, 265] e apontaram para uma possível associação do polimorfismo da LTA 252 A>G com o diagnóstico de AR. Optámos assim por alargar o estudo deste SNP a uma população de maiores dimensões (Parte III). Neste estudo caso-controlo que incluiu 388 doentes com AR e 269 controlos saudáveis, todos caucásicos, o alelo A (OR 1,325) e o genótipo A/A (OR 1,772) associaram-se de forma significativa com o diagnóstico de AR. Constatámos que este mesmo genótipo mostrou uma associação independente com a presença de dislipidémia (OR 18,3; IC 95% 2,1-94,8) e, entre os doentes não medicados com fármacos hipolipemiantes, os portadores de LTA 252 A/A apresentam valores de triglicéridos significativamente mais elevados e valores de colesterol total também mais elevados, embora a diferença nos níveis de colesterol não atingisse a significância estatística. Tendo em consideração que a LTA participa em diversos processos para além da inflamação, incluindo a homeostase dos lípidos e a formação das placas ateroscleróticas [248, 249, 266], estas observações estão em linha com a possibilidade de determinantes genéticas estarem associadas simultaneamente a uma maior susceptibilidade para o desenvimento de AR e a um risco aumentado de dislipidémia. A corroborar a hipótese de uma predisposição genética para a dislipidémia da AR estão os valores elevados de lípidos encontrados no soro de indivíduos que no futuro vêm a desenvolver AR, e que não são explicados por outras variáveis [267]. Este polimorfismo do gene da LTA afigura-se assim como um marcador de risco comum à AR e à aterogénese.

No quarto trabalho desta tese avaliámos as alterações hemorreológicas no LES e na AR em relação com a aterosclerose subclínica. Vários estudos epidemiológicos relacionaram perturbações hemorreológicas

com ocorrência de eventos vasculares isquémicos na população [268-270]. A viscosidade sanguínea e o fibrinogénio plasmático foram associados a eventos CV pelo menos com a mesma intensidade que os FR tradicionais [180]. Documentámos nas doentes com LES, assim como na AR uma agregação eritrocitária aumentada, diminuição da deformabilidade dos eritrócitos e diminuição da viscosidade sanguínea comparativamente com controlos saudáveis (Parte IV). Estas alterações correlacionam-se com dados demográficos, parâmetros inflamatórios e FR CV. A doença activa e a presença de síndrome metabólica concorrem de forma sinérgica para o aumento da agregação e diminuição da deformabilidade eritrocitária, criando um ambiente propício a alterações microcirculatórias e disfunção microvascular. As perturbações da microcirculação são uma complicação documentada no LES e na AR, mesmo na ausência de doença macro-vascular [271]. Verificámos que os doentes com anticoagulante lúpico circulante e os que têm RF IgM ou ACPA são os que apresentam valores mais elevados de agregação eritrocitária. Este subgrupo de doentes é também aquele que tem maior risco CV [111, 272], podendo o aumento da agregação eritrocitária contribuir para esse risco acrescido. Nas mulheres com lúpus ou com AR a aterosclerose subclínica associa-se a alguns parâmetros hemorreológicos. Documentámos a associação entre a agregação eritrocitária aumentada e produção de NO eritrocitário diminuída com a presença de placas carotídeas em análises univariadas. Em análise multivariada a viscosidade sanguínea (OR 3,98; IC 95% 1,23-12,83) e o NO eritrocitário (OR 0,16; IC95% 0,05-052) revelaram-se preditores independentes da espessura aumentada da íntima-média da carótida. A associação entre viscosidade sanguínea e IMT tinha sido anteriormente descrita em indivíduos do sexo masculino sem doença inflamatória. No presente trabalho pudemos documentar pela primeira vez esse efeito em mulheres com doença reumática inflamatória.

Os mecanismos pelos quais as perturbações hemorreológicas contribuem para a aterogénese e eventos CV poderão ser diversos. As interacções adesivas entre os leucócitos e o endotélio podem ser afectadas pela tendência de agregação dos eritrócitos. Por outro lado, a viscosidade sanguínea pode contribuir para a aterogénese através de um efeito mecânico na parede arterial. Apesar da aterosclerose ser uma doença inflamatória generalizada, as lesões vasculares não se distribuem de forma aleatória, mas localizam-se preferencialmente em locais onde há perturbação do fluxo sanguíneo, salientando o possível efeito das forças de cisalhamento para os distúrbios do endotélio. No LES e na AR as células endoteliais apresentam um fenótipo activado devido à inflamação sistémica, o que as torna provavelmente mais susceptíveis a outras noxas, incluindo o efeito deletério da viscosidade sanguínea. Embora as doenças inflamatórias cursem com viscosidade sanguínea inferior aos controlos em resultado de um hematócrito mais baixo, este parâmetro mostrou uma associação independente e positiva com o IMT carotídeo destes doentes. Na presença de síndrome metabólica a viscosidade sanguínea aumenta, sublinhando a relação entre a inflamação, os FR CV tradicionais e as características reológicas do sangue. Comparativamente à AR, as mulheres com LES apresentam um perfil hemorreológico mais desfavorável, caracterizado por um aumento significativo da viscosidade sanguínea e das concentrações de fibrinogénio.

Por último analisámos as alterações vasculares precoces através da determinação dos níveis séricos de biomarcadores vasculares e do estudo da função endotelial realizado por tonometria arterial periférica (Parte V).

Constatámos que as doentes apresentam níveis séricos elevados de sICAM-1, trombomodulina (TM) e factor tecidular (TF) comparativamente aos controlos, e que a elevação da TM e do TF

persiste após ajuste para co-variáveis e potenciais confundidores. Como esperado, os biomarcadores vasculares correlacionam-se com parâmetros inflamatórios e encontram-se mais elevados na doença activa do que na doença quiescente. Contudo, observámos valores de TM elevados mesmo nos doentes em remissão clínica.

Globalmente, as alterações vasculares precoces são mais pronunciadas no lúpus. Estas doentes apresentam um padrão distinto de biomarcadores vasculares, e as diferenças comparativamente à AR persistem mesmo após ajuste para factores de risco CV e outras co-variáveis. Nas doentes com LES encontrámos um marcado aumento do sICAM-1 sérico e da TM, enquanto a AR se caracteriza pela elevação dos níveis de TF. Estes achados são relevantes, dado que as moléculas de adesão podem representar uma ligação entre inflamação e aterosclerose. Foi anteriormente documentado o aumento da expressão de moléculas de adesão sobre lesões ateroscleróticas [273, 274] e a importância que os níveis séricos, em particular de sICAM-1, representam como factor de risco independente para aterosclerose e preditor de eventos CV [275, 276]. O facto do sICAM-1 e do fibrinogénio estarem mais elevados nas doentes com LES pode ser relevante para o início e progressão da aterosclerose, porque ao ligar-se ao ICAM-1 o fibrinogénio promove a adesão e migração transendotelial de leucócitos, um passo inicial na inflamação vascular [244]. Identificámos igualmente o fibrinogénio como um preditor independente de disfunção endotelial no LES e na AR.

A expressão da TM, uma molécula com propriedades anti-coagulantes, encontra-se diminuída durante o processo inflamatório, e a TM da superfície da célula endotelial é clivada e libertada para a circulação, pelo que os níveis séricos elevados desta molécula são um indicador de provável lesão endotelial [277]. Juntamente com o aumento do TF, um iniciador da via extrínseca da coagulação, geram um ambiente propício a complicações trombóticas.

Não encontrámos diferenças na função endotelial medida por PAT entre doentes e controlos, nem entre o lúpus e a AR. Contudo, na doença activa a vasodilatação reactiva é menor no LES do que na AR.
Em ambas as doenças a actividade inflamatória, mesmo ligeira, associa-se a uma probabilidade 3 vezes superior de disfunção endotelial. Adicionalmente, os doentes com LES apresentam um AIx mais elevado, o que indicia uma pior condição vascular. O maior risco CV dos doentes com lúpus é assim consubstanciado por estas alterações vasculares precoces mais pronunciadas.

CONCLUSÃO

A etiopatogénese da aterosclerose no contexto das doenças inflamatórias imunomediadas é multifactorial. No conjunto dos estudos realizados avaliámos factores de risco CV tradicionais, alterações da composição corporal, variáveis genéticas, marcadores de inflamação, biomarcadores vasculares e alterações hemorreológicas, e estudámos a função endotelial e a presença de aterosclerose sub-clínica por ecodoppler carotídeo em doentes do sexo feminino com LES ou com AR sem doença cardiovascular prévia conhecida. Foi o principal objectivo deste trabalho identificar factores que concorrem para a aterosclerose subclínica nas doenças reumáticas inflamatórias e desta forma contribuir para a sua prevenção.

Identificámos uma elevada prevalência de factores de risco CV modificáveis no LES e na AR. A actividade inflamatória e a terapêutica interagem com os FR CV e contribuem para alterações hemorreológicas que se revelaram importantes preditores de aterosclerose subclínica. O contributo da inflamação é essencial para as perturbações do endotélio e para a disfunção endotelial. É plausível que factores genéticos contribuam simultaneamente para a susceptibilidade para a doença inflamatória e para aterosclerose prematura.

Documentámos diferenças entre as duas patologias no perfil de FR tradicionais, nos mediadores da inflamação, nas alterações hemorreológicas, nos biomarcadores de activação endotelial, na rigidez da parede vascular e, em menor grau, na resposta vasodilatadora à isquémia. No seu conjunto as alterações vasculares são mais pronunciadas nos doentes com lúpus, o que está em linha com o maior risco vascular associado a esta doença.

A disfunção endotelial, assim como as alterações hemorreológicas, são mais evidentes na doença activa do que na doença em remissão, pelo que é expectável que mantendo um controlo estrito da actividade inflamatória seja possível reduzir o seu impacto negativo nos vasos.

Por fim, mas não menos importante, é a necessidade de controlo dos FR CV tradicionais nos doentes com doenças reumáticas, uma área onde existem grandes lacunas.

Em resumo, neste trabalho foram identificados diversos aspectos que contribuem para a aterogénese acelerada nas doenças inflamatórias sistémicas. Os resultados obtidos corroboram a importância de um controlo estrito da actividade inflamatória e dos FR CV associados. As diferenças encontradas entre o LES e a AR poderão contribuir para uma avaliação clínica e intervenção terapêutica mais direccionadas.

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